MISION PERMANENTE DE CUBA
ANTE LAS NACIONES UNIDAS
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The Permanent Mission of Cuba to the United Nations presents its compliments to the Secretariat of the G-77 in its capacity of Secretariat of the Perez-Guerrero Trust Fund for Economic and Technical Cooperation among Developing Countries, and has the honor to enclose herein the Final Report 2003-2004 of the Project entitled "Increase in sugar production by microbiological inhibition of the Leuconostoc spp. and other bacteria", headed by the Cuban Institute for Research on Sugar Cane By-products (ICIDCA).

The Permanent Mission of Cuba to the United Nations avails itself of this opportunity to renew to the Secretariat of the G-77 the assurances of its highest consideration.

New York, September 19th, 2005.

To the Secretariat of the G-77
New York

Misión Permanente de Cuba
O.N.U.

PGTF
THE PEREZ-GUERRERO TRUST FUND FOR ECONOMIC AND TECHNICAL COOPERATION AMONG DEVELOPING COUNTRIES

FINAL REPORT
2003-2004

Code of the project: INT03/K09

Title of the project: "Increase in sugar production by microbiological inhibition of the *Leuconostoc* spp. and other bacteria" (ISPLI)

Coordinator: Cuban Institute for Research on Sugar Cane By-products (ICIDCA).

Description of the consortium:

ICIDCA	ICIDCA	Cuba
EEAOC	EEAOC	Argentina
	IBT/UNAM	Mexico

Head of the Project: Dra. Georgina L. Michelena Alvarez, ICIDCA

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I. Organization of the ISPLI Project

a) Project objectives:

The research work aims at improving our knowledge of the control microbiological methods for microbial inhibition of the losses of cane sugar in the process of production of sugar. As immediate objective it is sought to design a biotechnical outline of interaction of microorganisms in order to sugar recovered increasing in the sugar mill and to quantify the reduction in the sucrose consumption and the formation of polysaccharides during the process of production of the sugar.

This objective will be reached completing the following stages:

1. Production of microbial metabolites with inhibitory action that act over diverse strains of *Leuconostoc* (emphasizing in the variety of autochthonous *Leuconostoc* from come each country involved in the project).
2. Dose, conditions and points of application definition of the microbial metabolites in the process of production of sugar.
3. Design of the technological outline that allows the establishment of the methodology of microbial inhibition for increasing sugar recovered.
4. Quantification of the decrease in the sucrose losses.
5. Contribution to the qualitative conception of the production of sugar and ecological sugar.
6. Improves of the industrial yields for decreasing of the production costs.

Therefore, this project will provide of biotechnical tools in the production of cane sugar, with the objective of:

1. Diminish the sucrose losses for formation of polysaccharides.
2. Improve the quality of the sugar formed in the crystallization process.
3. Diagnose the sucrose losses taken place in the factory by microbial effect.
4. Contribute to the scheme of ecological production of sugar with high added value in the international market.

In this project three grateful institutions of Mexico, Argentina and Cuba that will be in charge of developing the studies proposed for the definition of the biotechnical scheme will be taking part. Two sugar factories will serve as reference pattern and they agree on to give the necessary information and to evaluate the results of the inhibition process that it is implemented. This will allow to quantify particular and general characteristics in the proposed outlines and will facilitate to give a multiplier focus to the problem and to create a solution outline that adapts to any factory of cane sugar coming from any country, for what is also sought with the project that possesses an amplifier effect and to obtain a transferable technology toward any sugar country.

The expected results of the proposed research work will be applicable in a wide range of scenarios whose objective is to diminish the sucrose losses for microbial effect considering its economic effect in the decrease of the costs with respectful solutions to the environment.

b) Project Outputs.

The outputs of the project are:

1. Isolation and selection of *Leuconostoc mesenteroides* coming from the cane soil 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. Development of microbial metabolites with characteristic of inhibition of the growth of the *Leuconostoc mesenteroides* and of the dextrans production.
   Products with inhibitory characteristic of the *Leuconostoc* will be defined and developed until the commercial presentation, and therefore they will impact directly in the microbiological control of the sugar factories.

   It will be presented in publications. Commercial mark of the products and their properties are registered.

3. Design of a technological methodology for the application in sugar mill based on the microbiological interaction of the *Leuconostoc* and other bacteria with metabolites microbial, defining dose, conditions and application points.
   An invention patent will be written. Manual of Procedure will be written. This document could have comercial value.

4. Economic inform of the balances cost-benefit keeping in mind the cost of application of the biological products and their direct incidence on sugar recovered, quality of the grain of sugar and effect of the increase of the price of the sugar when being exempt of polysaccharides.

The integrative nature of the project will allow that these results lead to the achievement of the proposed to medium term objectives that is in definitive, the increase of the production in the sugar mill and the sugar efficiency.

c) Project Activities

The activities of the project have been distributed in 6 work packages, one of them being the coordination task. Each one of these packages is leaded by an institution in the project, but every partner participates in all work packages.

Work packages.

Work Pack.	Work package title	Leader	Month		Manpower (man-months)			
			Start	End	ICIDCA	EEOC	IBT/UNAM	Total
0	**Project Management**	ICIDCA	1	24	2	1	1	4
1	Strains isolation from cane soils and sugar process	EEOC	1	6	2	2	1	5
2	Microbial metabolites production from fungus and bacteria with inhibitory characteristic	ICIDCA	1	6	8	2	1	11
3	Microbial metabolites production from actinomycetes with inhibitory characteristic	EEOC	1	6	3	5	1	9
4	Study of inhibitory effect on *Leuconostoc* and others bacteria	EEOC	7	17	4	4	1	9
5	Isolation and characterization of *Leuconostoc* genes	IBT/UNAM	1	24	1	1	5	7
6	Preliminar study of industrial validation. Economical evaluation. Prefeasibility study.	ICIDCA	17	24	10	6	1	17
	TOTAL				30	21	11	62

The partners have been working in the work packages since the First Project Meeting.

d) Steering Committee of the Project

Coordinator:

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II. Meetings

There are changes in the meetings schedule because of the delay of project approval. The project technical work meetings have been schedule in the following dates and places:

Date	Objectives	Meeting place
June 2003	Kick-off meeting. Definition of individual tasks and final arrangements in work packages.	Tucumán, Argentina
May 2004	Progress evaluation.	Cuernavaca, Mexico
December 2004	Final meeting. Discussion of results and final reports.	Havana, Cuba

a) KICK-OFF MEETING

Visited country: Argentina
Foreign institution: Experimental Agro industrial Station Obispo Colombres (EEAOC).
Date: June 1 - 16/ 2003

The visit took place in the mark of the project INT/03/k09 " Increase of Sugar Production for Inhibition of the *Leuconostoc spp.* and other bacteria" (ISPLI) and responding to the invitation of the Dra. Graciela Cerutti, head of the Microbiology Laboratory of the EEAOC.

The objectives outlined for the meeting were:

1. Meeting of beginning of the project:
2. Definition of activities of each part (Mexico, Argentina and Cuba), establishment of the dates of delivery of results, responsible for each result, discussion of the budget.
3. To Assay with preliminary designed products designed the inhibition of the *Leuconostoc mesenteroides* for diminishing the sucrose losses in the industrial process.
4. To design of a technological diagram that allows to establish the methodology of inhibition microbial to increase the sugar yield.
5. To report Argentinean conditions involved with the problem of production of polysaccharides in the industrial process.
6. Exchange of experiences and literature inside the laboratory of microbiology of the EEAOC, other laboratories and in other academic marks.

The kick-off meeting took place in the Laboratory of Microbiology of the EEAOC among the days 3 and 6 of June with the presence of the Dra. Clarita Olvera Carranza for the Mexican part, Dra. Graciela Cerutti for the Argentinean part and Dra. Georgina Michelena for the Cuban part. Execution possibilities were discussed and the activities dates and responsible were put according was described in the chapter D of the project (ACTIVITIES).

In the mark of the meeting the following agreements were took:

1. The Mexican part took to its laboratory five strains isolated in Argentinean cane soil for its identification for technical of molecular biology and the determination of the polymer that takes place (levan or dextran).
2. The Cuban part will make arrive to the part Mexican five strains isolated in Cuban cane soil with the same end.
3. The Cuban part will send to the part Argentinean product inhibitor from *Streptomyces* for its evaluation in laboratory scale and industrial during the Argentinean campaign of the 2003.
4. The Argentinean part will send to the part Cuban results that they are obtained in these assays that will be carried out with the product.
5. Budget was discussed and it was decided the purchase interest of Argentina and Mexican part.

Later, between 6 and 16 of June, Dra. Georgina Michelena and Dra. Graciela Cerutti worked in the laboratory of microbiology of the EEAOC, assaying inhibitory effects of the product and proving its application. The results of this study are integrated in the WP4.

Other exchanges of experiences in the EEAOC and other academic marks was also carried out and next detailed.

EXPERIENCES EXCHANGE IN EEAOC AND IN OTHER ACADEMIC INSTITUTES

During the visit, Dra. Georgina Michelena met with the Engineer Geronimo Cárdenas, Assisting Director of the EEAOC for summarizing of a bilateral project with a vision amplificatory of the current project that it includes the good practices of industrial factory for the decrease in the sucrose losses for effect microbial.
"Diversified Biotechnology of the cane of sugar" conference was imparted in the EEAOC and had exchanges of experience, opening possibilities for future collaborations with the Dr. Atilio Castagnaro, head of Biotechnology department of the EEAOC interested in the studies of determination in the defense mechanism in plants of the jasmonic acid and the gluticid and the Dra. Alicia Ragout specialist in lactic acid and lactic bacteria of the PROIMI.
Also, relationships of collaboration and exchange of experience settled down with the Dra. Gracia Rivas Montes of the Biochemistry, Chemistry and Pharmacy Faculty of the National University of Tucuman in the topic of dextran derivatives for pharmaceutical use with the intention of establishing future exchange projects.
visits of scientific exchange in the Plant Pilot of Industrial Processes Microbiologic of Tucumán were done where a meeting with its director Dr. Faustino Siñeriz took place and the relationships of collaboration existent between this Institute and the ICIDCA were narrowed.
The Dr. Siñeriz made a kind contribution to the ICIDCA, consistent in patterns and enzymatic kits of determination of lactic acid and its isomeric valued in near 200 USD.
It was also contacted with the Dr. Raúl Vilariño of the Secretary of Environment of Argentina in Buenos Aires where they were defined Argentinean interests in the use of distillery vines in collaboration with Cuba. This contact was also carried out with the Engineer Juan Luis Fernández, Secretary of Services and Productive Activities of Tucuman.

b) Second Work Visit

Visited country: Mexico
Foreign institution: Institute of Biotechnology, UNAM, Cuernavaca
Date: April 15-May 12, 2004
The visit took place in the mark of the project INT/03/k09 " Increase of Sugar Production for Inhibition of the *Leuconostoc spp.* and other bacteria" (ISPLI) and responding to the invitation of the Dr. Agustín López Munguía, director of the Laboratory of Enzimology of the IBT.

The objectives outlined for the meeting were:

7. Meeting of pursuit of the project.
8. Isolation and characterization of the genes of the Leuconostoc
9. Characterization of enzymes producer of polysaccharides.

10. Exchange of experiences and literature inside the laboratory of enzimology of the IBT, other laboratories and in other academic marks.

The meeting of the project took place in the Laboratory of Enzimology of the IBT on 21-26 April with the presence of the Dr. Agustín López Murguía, Dra. Clarita Olvera Carranza for the Mexican part, Dra. Graciela Cerutti for the Argentinean part, MSc. Aidín Martínez and Dra. Georgina Michelena for the Cuban part. Annual Report 2003 of the project was discussed and the possibilities of execution of the tasks for the 2004.

In the mark of the meeting the following agreements were took:
1. To execute to the work package 5 about characterization of the strain and the production of the polysaccharides that take place.
2. To investigate in Mexico and Argentina types of inhibitors of investment of juice or disinfectant of the sugar process that is used in the industry.
3. To make economic valuations of the product developed in the mark of the project comparing it with the similar ones commercial.

Later, between April 26 and May 12, Dra. Georgina Michelena, MSc. Aidín Martínez and Dra. Clarita Olvera worked in the laboratory of enzimology of IBT in the characterization of enzymes producing polysaccharides. The results of this study are integrated in the WP5.
Other exchanges of experiences in the IBT and other academic marks was also carried out and next detailed.

EXPERIENCES EXCHANGE IN IBT AND IN OTHER ACADEMIC INSTITUTES
During the visit, Dra. Georgina Michelena met with the Dr. Jesus Rodríguez, Head of the Dept. of Biotechnology, of the Autonomous University of Cuahuila and the Dra. Yolanda Anaya, Assisting Director of the UAC to discuss the realization of bilateral projects or with international fund with a spraying vision of the current project and in collaboration with the ICIDCA that includes metabolites production, enzymes and use of technical of immobilization. The cycle of conferences was imparted "Biotechnology and Diversification of the Cane of Sugar" in the UAC and one had exchanges of experience, opening possibilities for future collaborations with the MSc. José Luis Martínez and the Dra. Irina Illyanova, for establishing new exchange projects.
It was also carried out a short visit to the UAM, Iztapala for upgrading the topics developed old collaborators of the ICIDCA like they are the Dr. Mariano Gutiérrez, Dr. Ernesto Favela and the Dr. Felipe López.

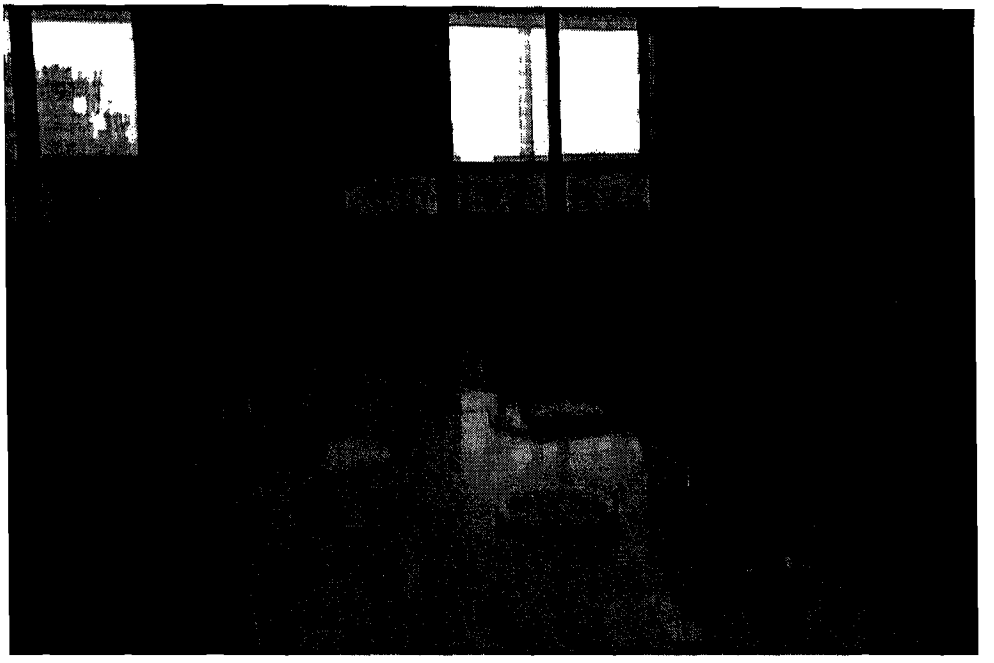
c) Final work Meeting. ICIDCA,. La Habana, Cuba

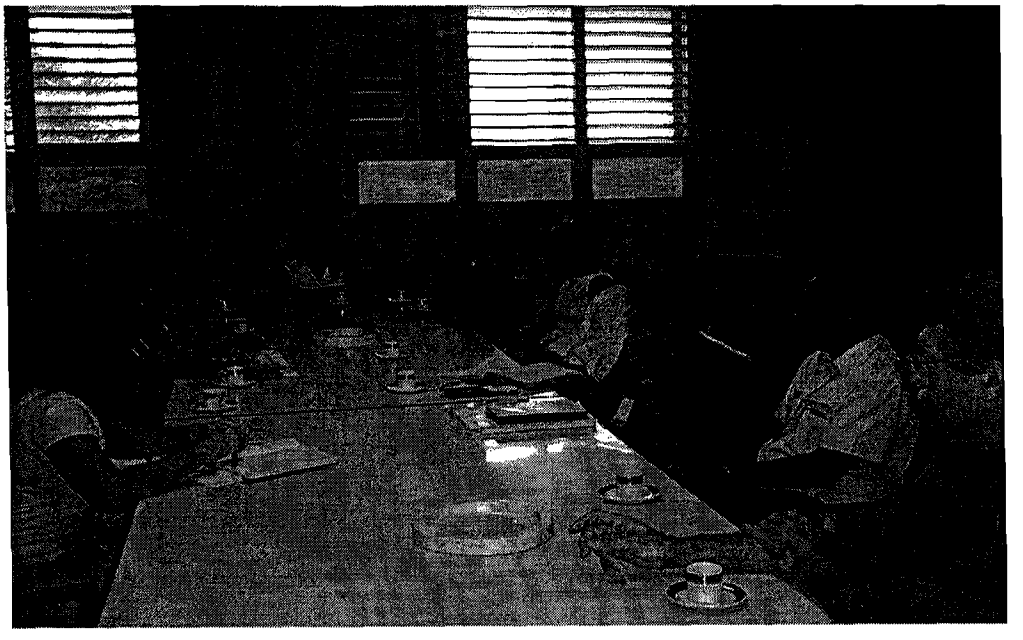
Country: Cuba
Institution: Research Cuban Institute on Sugar Cane by-products , ICIDCA, C. Habana
Date: December 4-10, 2004

Participants:
Dra. Georgina Michelena, Head of the project, ICIDCA

Dr. Agustín López Munguía, Mexican coordinator
Dra. Graciela Cerutti, Argentinan coordinator
Dra. Clarita Olvera Carranza, Mexican specialist
Lic. Aidín Martínez, member of the project, ICIDCA
Dr. Antonio Bell, member of the project, ICIDCA
Emilia Carrera, member of the project, ICIDCA
Lic. Jorge García, specialist of the MINVEC, Cuba, invited
Dr. Pierre Monsan, Francia, specialist invited
Ing. Esperanza Valdés, Head of Biotechnology Division, ICIDCA, invited
Dr. Amaury Alvarez, Head of Research Division, ICIDCA, invited
Lic. María Elena Díaz de Villegas, Head of Biochemistry Dept. ICIDCA, invited

The works sessions included the following activities:
1. Wellcome to all participants on behalf of ICIDCA
2. Presentation of the result of each institution: ICIDCA, EEAOC, IBT
3. Disscussion of the results
4. Words from Direction of ICIDCA about the project
5. Words from MINVEC about the project
6. Presentation of the new proposal to the Perez Guerrero Trust Fund including the Brazil participation (UNAERP) and some new cuban and brazilian industries.
7. Social event hosted by ICIDCA





III. Evaluation of the objectives of the ISPLI Project

All the objectives conceived have been achieved. Task are going on through the expected plan, although new ideas complementing the final aims have been arise and will be include in a new proposal for continuation.
The work team climate is very good among all participants. The contacts are stable and new ideas for bilateral and multilateral new projects are being under study. Some dissemination activities have been done.
In the meetings it was discussed, approved and write the continuation of the work in a new proposal Perez Guerrero Trust Fund presented including the participation of Brazil (University of Riveron Preto, Sao Paulo) and four new sugar mills. Some new relevants tasks will be done:

1. Optimization of the conditions of fermentation for the production of metabolites with characteristic of inhibition of the growth of the *Leuconostoc mesenteroides* and of the dextrans production.

2. Commercial mark of the products with inhibitory characteristic of the *Leuconostoc* and their properties are registered.

3. To generalize toward other sugar mills the use of the product. To extend validation of the product with a technological methodology for the application in sugar mill based on the microbiological interaction of the *Leuconostoc* and other bacteria

Some proposal for a CYTED, SETCIP and FAPESP Project will be prepared looking for other financial contributions.

National projects in each country are also supporting the project tasks. The collaboration of some institutions which are not part of the part of the project consortium is well valued. These institutions are:

- University of Riveron Preto, Sao Paulo, Brazil
- University Autonomous of Mexico, Iztapalapa, México
- University National of Tucumán, Argentina
- INDOTEC, Santo Domingo, República Dominicana
- University Complutense of Madrid, Spain

National Teams meetings
In every institution, technical work meetings have been carried out for fulfilling the task of the project. The relevant information on these meetings has been distributed among the partners.

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

Isolation and characterization of new dextran strains from cane soils and sugar process.

With the objective of the isolating of new strains from the intermediates of the sugar production as ace, cane juice, molasses, soil, etc, was carried out this study to determines the characteristics of the dextrans produced, compared with the argentine T6 strain and the acquaintance and standardized in the ICIDCA one, the *Leuconostoc mesenteroides* B/110-1-1 and defining biological methods against *Leuconostoc* that increase the sugar recovered. Samples of differents juice, molasses and soils were processed. They were inoculated in specific broths for the growth of *Leuconostoc*, composed by sucrose, yeast extract and salts. The isolation of three new strains was achieved, being observed to the microscope typical morphological characteristics of the *Leuconostoc* genus that is distinguished for its spherical cells, ace corresponds to *Streptococcaceae* to which it belongs.

Isolation and Identification of the Cuban strains

1. Collection of samples:
Samples coming from intermediate currents of the sugar mill and cane soils during the harvest sugar January - May of the 2003. The samples were taken in sterile flasks corresponding to the following currents:
Alcalinizaded juice (JA)
Clarified juice (JC)
Filtered juice (JF)
Blended juice (JM)
Primary juice (JP)
Physical molasses of the sugar mill "Argelia libre"
Physical molasses of the sugar mill "Majibacoa"
Physical molasses of the sugar mill "Jesus Menéndez"
Physical molasses of the sugar mill "Antonio Guiteras"
Piece of sugar cane coming from annexed cultivated area to "Antonio Guiteras"
Cane cultivated soil near to sugar mill "Antonio Guiteras".

2. Cultivation medium for *Leuconostoc* growth:
From the samples dilutions of 10^{-1} up to 10^{-4} were done. It was sowed in Petri dish 0.1ml of the direct dilutions in the surface of the substrate in solid means for isolation of Leuconostoc. The dishes were incubated to 30 °C during 48 h. The diplococcus colonies Gram was isolated - positive with formation of polysaccharides on its surface were isolated employing technique for grooves in Petri dishes with medium solid.
Later pre-inocule for the identification of the cultivation and the characterization of the strains were realized.

3. Identification of the strains : The inocule was carried out in Erlenmeyer of 50 ml with 30 ml of the medium for *Leuconostoc* incubating to 30 °C during 24 h. On the base of the kit API and biochemical reactions for *Leuconostoc* were carried out the identification of the strains.

I. Isolation and Identification of the strains:
Growths were obtained from samples of the different intermediate currents of the production of sugar, being detected colonies with characteristic morphological similar to the Leuconostoc as for: cells diploides in form of coconuts covered by polysaccharides of high viscosity.
Three strains were isolated that were named as C2, M5 and M3.

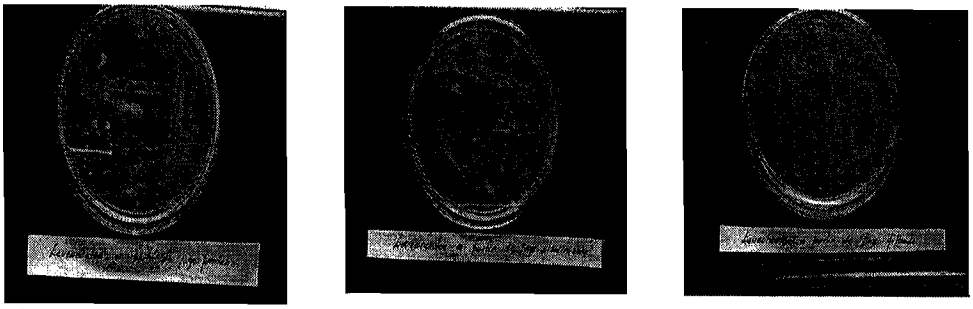


Fig. 1. Examples of isolation of new strains from sugar currents

By means of the system API 50 CHL (1998) of identification taxonomically was classified the new strains like L. *mesenteroides spp. mesenteroides dextranicum.*

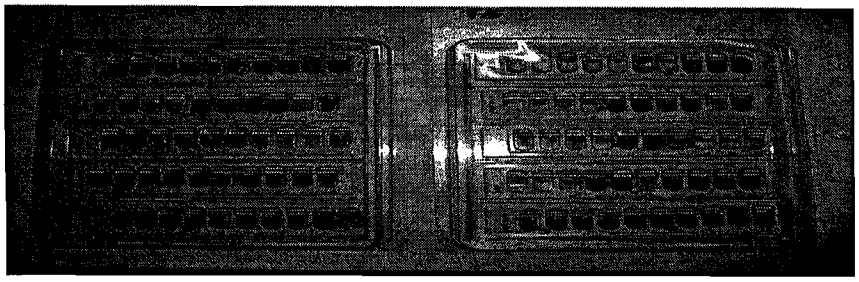


Fig. 2 Test of API applied to the new isolated strains. (Example)

From the point of view of this work, to find strains of Leuconostoc highly producer of dextrans in the industrial process of sugar is an unfavorable property, because it is indicative of more affectations, but from the point of view of dextran production to obtain derived and other products are favorable the isolation of strains highly producers.

On the other hand, it is important the isolation of new strains that present characteristic very diverse in the dextrans as a result of differences in the structure and the molecular weight. This aspect represents an important starting point for the development of derived products of the cane of sugar.

CONCLUSIONS

Three new strains were isolated in intermediate currents of the sugar process that were classified as L. *mesenteroides.*

Of the new isolated strains, M3 presented the biggest enzymatic activity, showing bigger activity that the patron ICIDCA.

The characteristic reological of the obtained dextrans is different should correspond to diversity in the chemical structures of the obtained polymers.

Isolation and Identification of the Argentinan strains

During the Argentinean harvest (May-October) three strains were isolated respectively from Actinomycetes and were denominated as MC1, MC2 and MC3, starting from cane of sugar and of juices of the industrial process. The strains were identified according to the diaminopimelic acid (DPA) using technical of chromatography in fine layer. The isolated Actinomycetes belongs all to the gender Streptomyces.

They were also isolated, 23 microorganisms of the gender Leuconostoc. The identification taxonomical was carried out according to the Manual of Bergey 9th Edition, using the system API for the profile of carbohydrates, growth to different temperatures, growth in different concentrations of Chloride of sodium and dextran production starting from sucrose using the mediums of cultivation of Garvie. Of the 23 isolated strains, those were selected that presented bigger speed of growth and dextran production in selective means with sucrose like source of carbon. The selected strains identified as Leuconostoc mesenteroides subsp. *mesenteroides 1K, 2L, 3A and 7B.*

a) Microbial metabolites production from fungus with inhibitory characteristic.

INTRODUCTION

Jasmonic Acid (AJ) is a regulator of the endogenous vegetable growth, synthesized in a natural way for a great variety of plants (Meyer, A. and cabbage., 1984); it belongs to the regulators of the vegetable growth (RCV) denominated jasmonates, being the most representative the jasmonic acid (-) - AJ and the (+)-7-isojasmonic acid ((+)-7-isoAJ) which are broadly distributed in the plants (Salisbury and Ross,1992).

The methylic ester of the AJ (AJ-me) was isolated for the first time as a main odoriferous constituent of the oils essentials of the flowers of *Jasminum grandiflorum* L (Demole and cabbage., 1962) and *Rosmarinus officialis* L (Crabalona,1967), reason why this compound is recognized as an important ingredient in the industry of perfumes and aromas (Koda,1992; Hamberg and Gardner,1992).

After the detection of the AJ-Met in *Artemisia absinthium*, grateful as a substances promoter of the senescence (Ueda and Kato,1980) and of the identified free acid as inhibitor of the growth in the pericarp of Corrupts (Dathe and cabbage., 1981), these compounds were able to attract the attention of many vegetable physiologists when being detected these compounds in different parts of the plants. It was isolated in 1971 starting from super of cultivations of the mushroom *Lasiodiplodia theobromae* and identified as potent inhibitor of the growth and senescence of the plants (Aldridge D. and cabbage, 1971).

Starting from the decade of the eighty, it could demonstrate in the mechanisms of defence of the plants (Kitahara T. and cabbage, 1991). It is known that the activation of genes in the plants for the pathogens attack or for mechanical wounds it causes the synthesis of defence substances like the proteases inhibitor and the tripsin in the damaged areas (Cohen, Y. 1993 ; Hamberg, M. and Gardner H, 1992 ; Miersch O., 1993). The application of the AJ in tomato plants and potato tubers has caused the accumulation of the proteases inhibitor (Yamagashi K., 1993) and in plants of rice it inhibits the germination of spores of *Pyricularia oryzae*, mushroom that causes the well-known illness as smut of the rice (Hamberg M., 1992).

The jasmonic acid (AJ) and their derived constitute a new substance group identified in a great variety of vegetable species (Meyer and cabbage., 1984), but of those that have not still been elucidated completely their physiologic functions (Staswick,1992; Hamberg and Gardner,1992).

The production of this regulator can be carried out through the extraction of the superior plants, for via chemistry or microbial. Until the moment the predominant way is the

extraction of natural sources in those that the AJ is at a low level, for what this product is placed in the world market at high cost, the prices oscillate among 300--400 USD/g quality reagent.
The knowledge on the production of AJ starting from microorganisms is even limited. Several species that are reported as producers of this compound, the most representative they are the strains of *Botryodiplodia theobromae* and *Gibberella fujikuroi* (Miersch O., 1989).

The studies carried out in cultivation liquid revealed that *Botryodiplodia theobromae*, fitopathogen fungu of tropical and subtropical areas that causes the rot of fruits and plants (Goos R., 1961), it is able to produce these types of substances as a result of their secondary metabolism with satisfactory yields (Miersch O., 1987).

The specific objective of this work was the evaluation and selection of fungus and bacteria producing of jasmonates and others metabolites in fermentation using diverse substrate.

MATERIALS AND METHODS

Microorganism
Brazilian strains 81, 83, c1, c2, c3, 3479 and 3184 obtained from biodiversity of the brazilian ecosystems were evaluated. Cuban strains 2334 and 1517 of *Botryodiplodia theobromae* from of the microorganisms collection of the National Institute of Fundamental Investigations of the Tropical Agriculture (INIFAT), Havana, Cuba were evaluated. Both Brazilian and Cubans cultivations were conserved in tubes with extract of malt-agar (Merck) to 4 °C.

Medium of cultivation
In the production of AJ the medium of cultivation Miersch (M-1) was used, it has sucrose like source of carbon and nitrate of potassium like nitrogen source. The medium modified was also evaluated . The compositions of medium used are described in the table 1.

Table I. Composition of the medium for the production of jasmonic acid

Components	M-1	M-2 (M1 mod)
Sucrose	50.000	50.00-
KNO3	3.000	7.500
KH2PO4	0.200	2.000
MgSO4.7H2O	0.200	0.600
KCl	0.100	0.300
FeSO47H2O	0.010	0.600
ZnSO4.7H2O	0.010	0.030
MnSO4	0.001	0.003
Na2MoO4.2H2O	0.001	0.003
CuSO4.5H2O	-	0.003
Yeast extract		1.000

Note: The concentrations are expressed in g/L.

Inoculums and fermentation conditions.
Petri dishes were used with 25 mL of extract of malt-agar and were inoculated from mycelium coming from the grown inclined tubes during 3 days, the Petri dishes were incubated by 5 days at 30°C. The cultivation medium was adjusted to pH 5.5 with NaOH (1N) and it was sterilized during 15 minutes at 120°C. 1/8 of mycelia dish was inoculated in erlenmeyers of 250 ml with 50 ml of medium, it was incubated at 30°C. Lapsed the time of fermentation was carried out the quantification of AJ.

Chemical analysis:

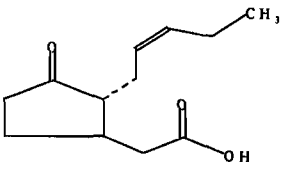
Biomass. The mycelia separated for filtration with filter paper Whatman 4, previously retarded, the residual dried off in stove at 60°C for 24 hours, the difference between the final weight and the initial was reported as formed biomass (%).
pH determination. The liquid super obtained in the determination of biomass was measured the pH directly in a digital potentiometer Crison.
Detection and jasmonatos quantification. The means of fermentation separated the mycelia for filtration to the hole, using filter paper Whatman N° 4. Aliquot of 5 ml of the filtered cultivation they were adjusted to pH 3.0 with HCl(4M) and extraction with ethyl acetate (1:1) was done. The fractions containing the AJ became dehydrated with anhydrous sodium sulfate and they were taken to dryness by rotoevaporation at 50°C. For the determination of the AJ and related compounds the technique of thin layer chromatography was used and its quantification by gaseous and liquid chromatography of high resolution.

RESULTS AND DISCUSSION

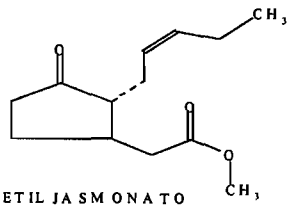
Implementation of methods for the detection and quantification of the production of jasmonates in the cultivations of B. *theobromae.*

Chemical properties of the jasmonates:
The jasmonates are compounds of the ciclopentan type with a cetonic or hidroxil group in the position of the carbon 6 and 2 ' cis - pentenil or another substitute alkilico in the position of the carbon 7.
The AJ presents two enantiomeres and two you form diastereomerics with the presence of two centers quirales in the ring of the ciclopentanone or the ciclopentan. It is known of the natural transformation in the way cis of the AJ and their compounds related to the form trans by 7 - epimerization during the isolation procedures and other treatments, being a balance molar among two forms trans to cis of 9:1.

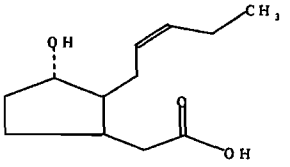
They are shown in the figure the structures in that naturally the jasmonic acid appears (AJ) and its related compounds.



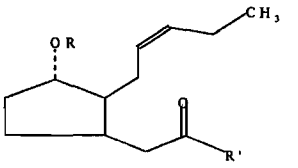
ACIDO JASMONICO



METIL JASMONATO



ACIDO CUCURBICO



R = β-D-glucosa
R' = OH

ACIDO O-β-D-GLUCOPIRANOSILCUCURBICO

R = H
R' = triptófano

N-(+)-CUCURBINOIL-S-TRIPTÓFANO

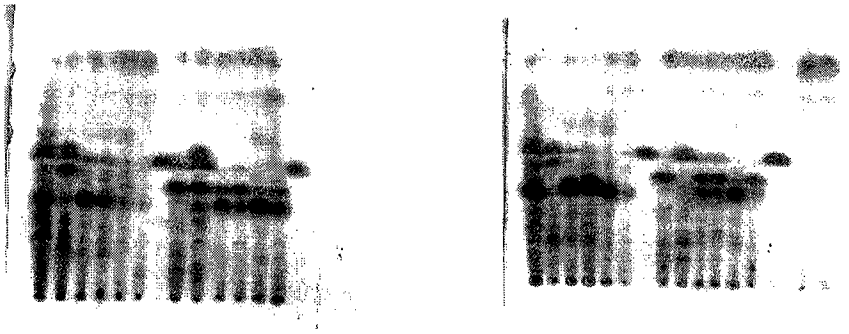
Thin layer chromatography (TLC)
The thin layer chromatography has been frequently used for the purification and jasmonates identification. The silica gel is the commonly used support and in other alumina can be used too.
The jasmonates can be detected by TLC with reagents, however they are not detected like stains against the fluorescent green base because they do not show significant adsorption to the UV.

Keeping in mind these chemical properties a form of detecting the jasmonates in TLC is for the use of the one revealed with reagents, for example with the reagent of Anisaldehido, with vainillin solution in concentrated sulfuric acid, with solution of potassium perganmanate to 1% or with exhibition to Iodine vapors.
These compounds are also detected with radiations UV like fluorescent stains after having been spreads with a solution of sulfuric acid - ethanol followed by heat at 130°C for 5 min.

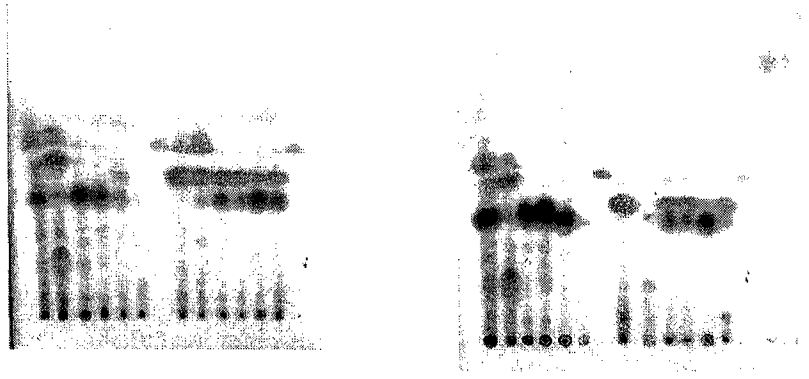
Methodology
Preparation of the sample
To 5 ml of the fermentation broth obtained for via microbial, using wild strains of *Botryodiplodia theobromae*, previously filtrate and centrifuged, is adjusted the pH at 2.8 - 3.0 with a solution of HCl (Strzelczyk and others, 1984).
It is carried out an extraction with ethyl acetate that is repeated 3 times, with 5 ml in each occasion.

2. Chromatography system:

Mobile phase: Cloroform:etyl acetate:acetic acid (40:10:5:1)
Stationary phase: Silica gel 60 G with an high de 0.25 mm.
Pattern of jasmonic acid.
Developers
Anisaldehyde: it mixes of acetic acid - sulfuric acid - anisaldehyde (100:2:1)
Ethanol-sulfuric acid



TLC 6to y 8vo day. Anisaldehyde



TLC 6to y 8vo day. Ethanol-sulfuric aci

Order of application of the samples: Cuban strains: 2334, 4 and Brazilian: 81, 83, 3184, 3479 (medium M-1) and pattern of AJ, in the same order (medium M-2) and pattern of AJ. From left to right 6to. and 8vo. day.

The quaternary system of MP selected is reported and their derived respective and a separation of the components of the samples was obtained applied with a resolution bigger than one.
With the developer of Anisaldehyde some authors report values of Rf 0.56 others of 0.70 for the AJ under these same conditions. The coloration of the stain of Rf agrees 0.52 with the one reported for this developer and the pattern of jasmonic acid, also appearing other stains in 0.42 and 0.37 in most of the analyzed strains, being atypical the case of the strain 1517 that it shows a stain very next Rf 0.49 to the stain of the jasmonic.
The spectrum of stains obtained with the developing ethanol - si TLC 6to y 8vo. day ethanol-sulfuric obtained with anisaldehyde.

Conclusions

The system of MP selected it offers a good resolution in the separation of the stains.
It is proven the presence of jasmonic acid in all the strains analyzed with bigger intensity in the strains 2334 and 1517.
According to the obtained Rf and comparing with Ueda (1994) the presence of cucurbic acid could be appreciated.

Gass chromatography (GC)

It was carried out previous "derivatization" with diazometane in situ obtained through the reaction of the methanol - sulfuric acid. A chromatograph Hitachi model was used 163 applying a detector of flame ionization (FID, for its initials in English), columns of glass of 3m x 3mm, packed with 3% of OV-225 CHROM-Q have more than enough (Meshes 100-120). As gas transporters argon was used with a flow of 30mL/min. The temperatures were regulated at 230°C in the injection and 190°C in the column.

Liquid Chromatography of high resolution (HPLC)

A chromatograph HPLC SHIMADZU was used (LC-10AD) with a column in reverse phase C18 Supelcosil (25 cm x 4,6 mm goes, 5(m) detecting of longitude of variable wave. Um flow of 0.85 ml/min, longitude of wave 210 nm and solvent MeOH: acetic acid 0.1%, 40:60 were used. The injection volume was of 20 uL.
The dried sample is dissolved in 1 ml of the mobile phase and 20 uL is injected to the team. The quantification of the AJ is analyzed by comparison of the picks with the curve pattern obtained for a range of values where they are the concentrations of the studied samples and they are processed by the software Biocrom for chromatography version 1.1.

Microorganism

Brazilian strains 81, 83, c1, c2, c3, 3479 and 3184 obtained from biodiversity of the brazilian ecosystems were evaluated. Cuban strains 2334 and 1517 of *Botryodiplodia theobromae* from of the microorganisms collection of the National Institute of Fundamental Investigations of the Tropical Agriculture (INIFAT), Havana, Cuba were evaluated. Both Brazilian and Cubans cultivations were conserved in tubes with extract of malt-agar (Merck) to 4 °C.

In summary, it was observed better productions with the strain 2334 in M-1 and M-4, with the 1517 in M-2, and with the strains Brazilian 3 in M-4 and 83 in M-5.

In the Fig. 5 are shown some of the differences described at the previous table:

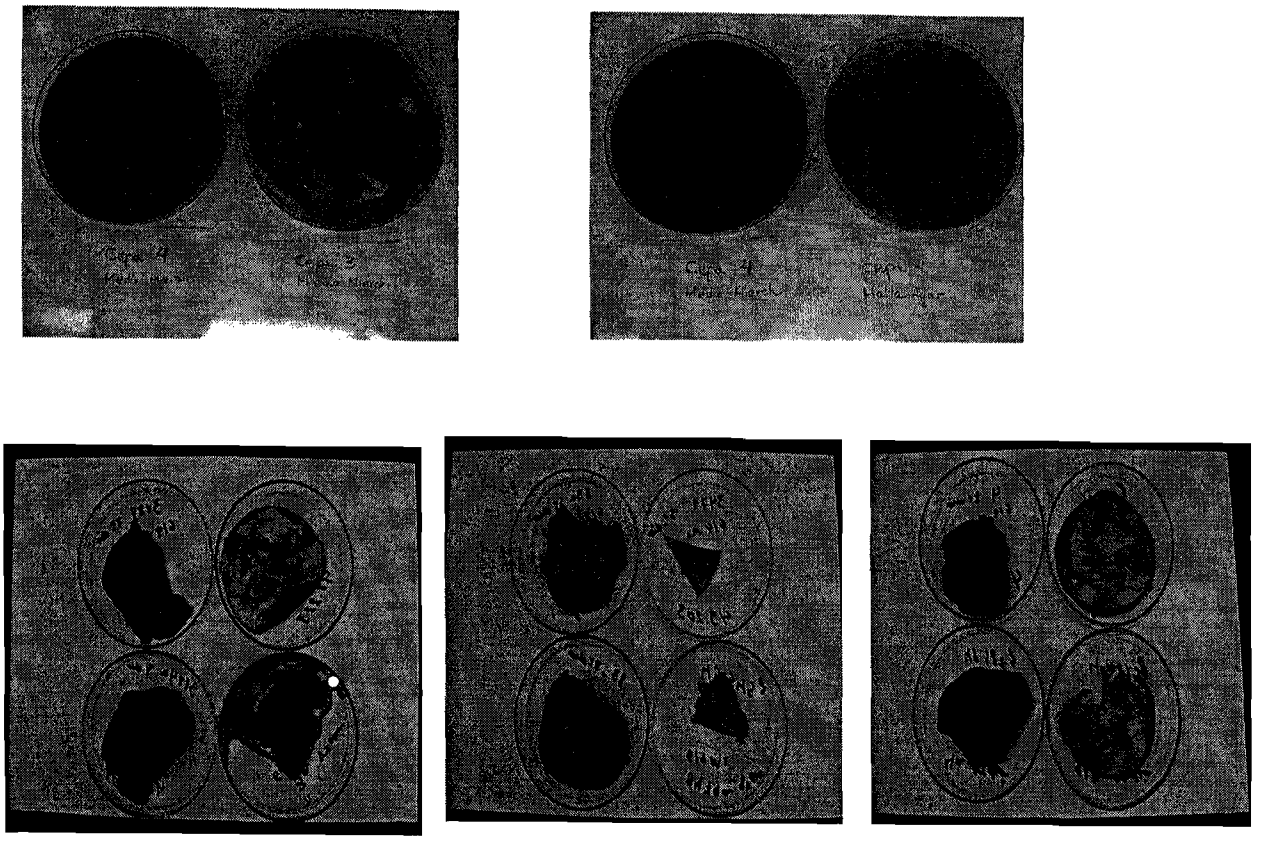


Fig. 5 Morphological differences among strains of B. *theobromae* in solid medium (above) and liquid (below)

As it can be observed strains in different mediums grow different, being shown a tendency to grow whiter in malt-agar and but black in Miersh (M-1) for the generality of the strains.

The liquid cultivations gave remarkable differences so much during the growth as the coloration of the raw ones, being observed the formation of a red pigment of a lot of intensity in strain 3 Brazilian. This pigment could be separate in a phase emulsified water - ethyl acetate during the extraction of the jasmonic acid.

Fig. 10 shows the result of the extraction with acetate of the strain 1517 (blue) and the pattern of AJ (red). Perfect coincidence is observed between the production of this strain and the corresponding com the pattern of AJ at 130 mm.

The Fig. 10 b shows the spectrum of the application of the extraction in acetate of ethyl of raw of the strain 1517 (rose) and the extractions of the strain 81 to the 10 days of fermentation and the 6 days of fermentation. Coincidences in the picks were observed of around the 50 and 180 mm, differing at the pick of the 81 in120 mm and 1517 in 130 mm and that it could be thought according to their polarity cucurbic for 81 to be more polar than the AJ.

(to) (b)

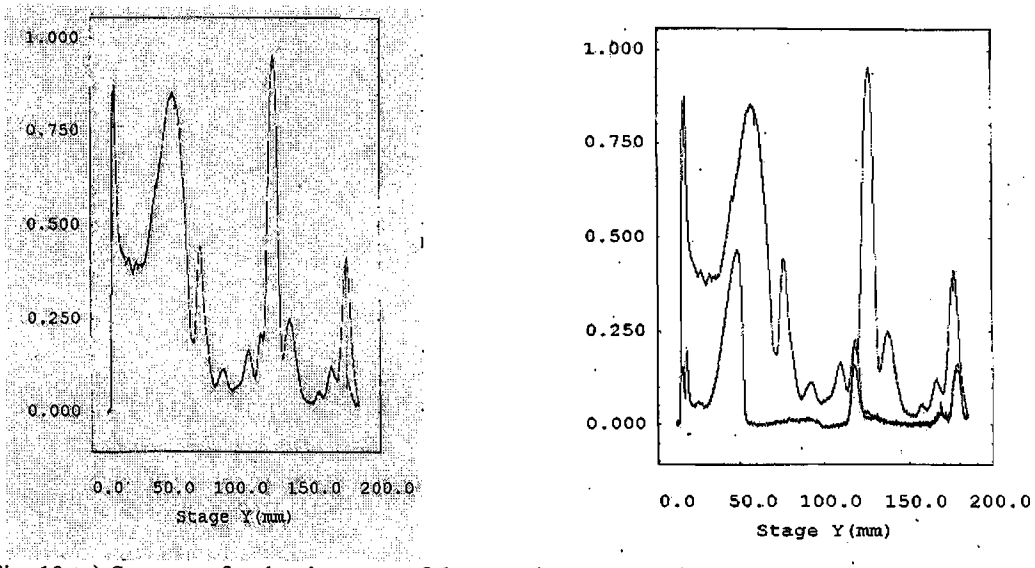


Fig. 10 to) Spectrum for densitometry of the organic extract of the strain 1517 (blue) and the pattern of AJ (red), b) organic extract of the strain 1517 (rosy), organic extract of the strain 81 to the 10 days of fermentation (blue) and organic extract of the strain 1517 (red).

The obtained result pleads favourably for the use of the cane broth like alternative of industrial substrate, mainly in the case of Brazilian strains.
The wealth of this source of carbon comes certain from the point of view fermentative for its high content of salts, sugars and substances probiotics, especially complex substances as amino acids, nucleic acids, vitamins, etc. that they represent a saving biosynthetic of the microorganisms.

CONCLUSIONS

1. Methods of characterization were implemented by TLC, HPLC and detection CG and quantification of extracted AJ of broths fermented by B. *theobromae.* It was selected composition of mobile phases and phases and conditions appropriate chromatographys to achieve high resolutions. The techniques mounted in the three cases showed high reproducibility and accuracy.
2. It was determined the speed of growth of the seven Brazilian strains and the two Cuban in medium of malt-agar and M-1. It was observed high speeds of growth in all the strains excepting the c1 and c2. The best results were for the strain 81, 3184 and c3 of Brazil and for 2334 (3) of Cuba. Morphologically differences were observed among the growth among strains and among medium, being a tendency to grow whiter in malt agar and blacker in M-1.
3. The curves of growth were determined, pH, consumption of sugars and production for all the strains in the means M-1 and M-2. The strains 2334 and 1517 were the best producers, achieving concentrations among 400-700 mg/L. Of the Brazilian strains the biggest concentration was reached by the strain 3 maximum com of 80

mg/L. This selection of Brazilian strains was carried out with 7 strains. It is necessary to increase the number until 30.

4. The presence of other products of more polarity that the jasmonic so much in the techniques for TLC, for readings densitometries in TLC preparative like for HPLC that could indicate presence of cucurbic acid and others derived. This production was outstanding in the strains Brazilian 3479 and 3 being reached maxima of 700 mg/L. It is necessary to highlight that the cucurbic acid has similar biological properties that the jasmonic and its price is of 1100 USD 50 mg, that which converts it in a metabolite of a lot of interest, could even be planned to direct investigations toward the preferential production of this metabolite. Preferably, it would be hoped to find strains producers of JA like majority metabolite and other producers of CA like majority.
5. The productions using cane broth gave concentrations smaller than JA in the Cuban strains that the obtained in M-1 and M-2, however showed bigger productions in the case of the strain 3 Brazilian in M-4 and 83 in M-5. These results plead favourably for the continuation of the studies fermentative using intermediate currents and sub-products of the sugar industry.
6. An strong pigment red was observed in the strains 3 of Brazil and in smaller measure in the 2334 of Cuba. The obtained spectrum of these raw ones showed two picks of absorption in the visible region one on the 400 nm and 540 nm. The production of this pigment was stimulated with light and with DMSO and the EDTA. The obtained spectrum and the reports of the literature suggest that it can be a antocianine.
7. In a general way, the capacity that has *Botryodiplodia theobromae* (coming from different biodiversities and continental regions) of producing the JA in very superior concentrations (more than 20 times) to the reported in plants and it was validated a methodology of fermentation and analytic methods for its quantification were demonstrated.
8. It was demonstrated an integrative character of methodologies and strains used in different countries, that which infers to the project the possibilities to multiply in other regions of the continent, inferring an attractive possibility of technological transfer to the project toward other countries.

The effect of the strains of Actinomycetes was studied about the growth of the microorganisms of the gener Leuconostoc using qualitative methods between solid, semiquantitative (diffusion in agar) and quantitative (Determination of the Inhibitory Minimum Concentration-IMC). For these tests means of selective cultivations were used as sucrose agar, SC agar (casein-starch agar) and YEME (yeast extract, extract of malt, glucose and sucrose). The obtained results showed that Streptomyces MC1, MC2 and MC3 produce inhibition of the growth of all the strains of selected Leuconostoc and studied between SC agar and YEME.

It was also demonstrated, by means of the diffusion method in agar that the super of cultivations of Streptomyces MC2 inhibits the growth of L. *mesenteroides* 1K and 3A in these results in liquid means with sucrose 10%

Strains of isolated actinomycetes of sugar have inhibitory capacity on growth and dextran production for *Leuconostoc.* This action is manifested at level of sucrose metabolism, affecting consumption of the same and production of the polysaccharide dextran. The biggest effect inhibitory 87.32% was observed in presence of the super of the strain MC2 without diluting. These results show the potential actinomycete utility in the sugar industry with the purpose of increasing the yields in sucrose obtained during the industrial process by reduction of losses originated by the production and dextran presence

Study of the inhibitory effect on the Leuconostoc and other bacteria.

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INTRODUCTION

The sucrose loss and the dextran formation are associated with the deterioration of the sugar cane. The produced sugar with deteriorated cane has a high dextrans content and it does not gather the acceptability conditions for its employment as raw material in the elaboration of some allowances. During years this it has been a problem that the industry has faced, becoming a challenge improving the quality of the sugar.

The *Leuconostoc mesenteroides* is the microorganism that synthesizes the dextransucrase and this in turns the dextran. It has been demonstrated that it can invade the fabric of storage of the sugar cane before the crop, entering for the cracks (3).

The physical and chemical characteristics of the sugar cane juice make of this an excellent basis for the development to the microorganisms. The metabolic characteristics of the microorganisms allow him to degrade the present sucrose in the juice and to form and to incorporate to the same metabolites like the lactic acid, acetic, ethanol, mannitol and polysaccharides like dextrans and levans (1).

The incidence of this phenomenon becomes remarkable in the mill area, where a fall of purity is observed from the primary juice to blended juice that can arrive to two integers for a high incidence of the bacterial degradation.

To counteract this fact, are the usual practices the exhaustive cleaning of the area of mills to where it passes the juice with hot water and vapor and germicide use later.

At the moment diverse chemical substances are employees as germicides and considered

as sanitization agents for sugar mills. Among them they are the halogens, organosulfurades and quaternary ammonium compound (2). However, few sanitization agents of biological origin have been proven in the sugar industry.

According to the analysis carried out by International Organisms and other institutions the affectation that takes place in the economic losses for the microbial action it can be exemplified with the following data:

Valuation of the losses for effect of the polysaccharides (data of GEPLACEA, 1987 in harvest of 5 millions)

1. Losses of 24 000 t of sugar refine and 11 000 t of raw sugar.
2. Increase of 2 800 t of petroleum in the refinement
3. Affectation of sugar quality. Tax for dextran content from 1.5 to 3.5 USD/t

Objective of the work:

In this work the effect of different disinfection methods is presented on the juices of the cane, in the microbial tenor decrease and the indexes of purity, that which impacts directly in a higher yield of sugar

MATERIALS AND METHODS

a) Effects in the *L. mesenteroides* inhibition for different methods to laboratory level and bank.

Microorganism

It was used the strains of L. *mesenteroides* subsp. mesenteroides 3A, 1K, 2L, 7B of the EEAOC Collection Cultivation, Argentina and B/110-1-1, B/110-1-2 and B/110-1-3 of the ICIDCA Collection, Cuba, which was conserved in tubes lyophilized. The microorganisms were isolated and identified from sugar cane and juices during the sugar harvests of Argentina (may-october) and Cuba (january-may).

As strains metabolites producers with effect antimicrobian were used: *Streptomyces* MC1, MC2 and MC3, *Botryodiplodia theobromae* 715 and 1517 and *Pseudomonas* sp. PSS.

The denominated inhibitor S was obtained composed by the copper salt of the jasmonic acid with other preservatives.

Metabolites Production with inhibitory effect:

Streptomyces was grown between casein-starch with a composition in (1L): starch 10 g, casein 1g, K2HPO4 0.5 g and YEME (in 1L): yeast extract 3 g, peptona 5 g, extract of malt 3 g, glucose 10 g and sucrose 340 g, during 48 h in shaker to 30 °C. The super was obtained by centrifugation to 10 000 rpm, during 30 minutes to 4 °C.

The jasmonic acid from B. *theobromae* was obtained media the cultivation Miersch (11-3) that uses sucrose source at the carbon and nitrate of potassium as source nitrogen. 10 fragments miceliales of 8 mm were inoculated (obtained starting from seeds carried out

in Petri dishes, in erlenmeyers of 5 000 ml with 1 000 ml of cultivation media Miersch), it was incubated at 30°C during 15 days. The fermentation media it was separated the mycelia for filtration to the hole, using filter paper Whatman N° 4. Aliquot of 5 ml of the filtered cultivation they were adjusted to pH 3.0 with HCl (4M) and they underwent extraction with ethyl acetate (1:1). The fractions containing the AJ became dehydrated with sulphate of anhydrous sodium and they were taken to dryness by rotoevaporation at 50°C. For the determination of the AJ and related compounds the technique of gass chromatography was used (3).

The Pseudomonas sp. PSS was grown in a media with glutamic acid and salts to 30 °C. The separated the biomass for centrifugation and dried off had dried off by aspersion it has more than enough ammonium sulfate.

Methods in solid media: to determine the antibiotic effect of the metabolites, agar diffusion technique was used (4). The detection of the antibiotic activity was determined by the technique of the poisoning of the means of cultivation with AN (bacteria)

Technique: The sterile filtrate of AJ was used, using membrane milipour of 0.22 (m, of concentration 246mg/L. Concentrations of the biological broth were evaluated, 2, 10, 15, 30, 60, 100 and 200 ppm, added in 20 mL of the cultivation means used in the antibiotic determination, for a re!ationship of 50%.
For the bacteria was carried out to the qualitative analysis, using a sample of AJ of 200 ppm.
The cultivation medium (witness and poisoned) was put in Petri dishes of 5.5 x1.5 cm for triplicate.

Methods in liquid media: medium was compound by sucrose 20 g/l, yeast extract 6 g/l, Na_2HPO_4 5 g/l and salts to study the inhibition of the growth and dextran production for the strains of *Leuconostoc*. The growth was determined by absorbance to 540 nm. The sucrose consumption was determined by HPLC and dextran according to the phenol-sulfuric method (5).

Effect of the inhibitor S on the present purity in the juices in the industry.
For the realization of this experience 10 liters of the dilute product 1:1 were applied in a divided pulse in the first and sixth mill, repeating the application to the 30 minutes.

The capacity of mill of the mill station is of 260 T/h, for a dose of approximate application of 0.007%. They took samples before the application, immediately after the application, to the 5 minutes of applied, to the 5 minutes after the second treatment and to the 30 minutes of the second treatment. Microorganism count was determined (yeasts, bacteria and *Leuconostoc*), as well as they were carried out pH measurements, pol and purity for the determination of the stability of the juices.

The times of contact settled down was the time out that can remain retained the juice mixed in the sugar factory before applying the action of the heat in the clarification process. This parameter can vary according to the operative characteristics of each factory. In this study were considered times of contact of 1, 5 and 30 minutes.

The number of viable cells (UFC/ml) it was determined using selective cultivation medium to establish the effect microbial of each one of the products in study on the total microorganisms (nutritious Agar), producing of polysaccharides (Yeast extract - Glucose-Agar) and eucariotes (yeasts and mushrooms, Malt-agar). The incubation was carried out to 37 and 30 °C during 48 h. The experiences were carried out for triplicate. The inhibition% was defined as% Inhibition = (UFC/ml inicial - UFC/ml final) / UFC/ml inicial x 100

RESULTS AND DISCUSSION

a) Effects in the inhibition of the *L. mesenteroides* for different methods to laboratory and bank level.

Study of inhibition of super of Actinomycetes on Leuconostoc.

It was determined that the super of the *Streptomyces* MC2 inhibited the growth of *L. mesenteroides* 1K showing the biggest inhibition halo (8mm) with the super of 48 h of cultivation. The biggest inhibition in the strain 3A was obtained with the super of MC2 120h. These results were confirmed following the growth of both strain of Leuconostoc between liquid more 10 sucrose%, where it was observed that the effect of the Streptomyces on the Leuconostoc is not only manifested on the development but also on the sucrose consumption and the dextran production. In order to quantifying the observed effect, for the method of IMC, it was determined that the same one falls as it increases the dilution of the super, it does not depend on the pH and it is manifested on the metabolism of the sucrose of the sensitive strains. *L. mesenteroides 3A* are able to consume 62% of the present sucrose in the means of cultivation liberating glucose, fructose and mannitol. The addition of the super of Streptomyces MC2 on a cultivation of this strain produced a reduction of 85% on the sucrose consumption and it diminished the dextran formation. In the Table 1 these results are observed.

Table 1. Sucrose consumption and dextran production for L. *mesenteroides 3A to the 6 h of cultivation.*

Super Streptomyces MC2	% consumed sucrose	mg/ml dextran
Control	100.00	3.57
Without dilution	1.67	2.00
Dilution ½	10.60	2.67
Dilution 1/4	44.69	3.02

The study of inhibition of the super of Actinomyces between liquid at level of plant pilot as it is shown in the Fig. 1, it showed that inhibition of the growth of the Leuconostoc

exists when dose of the super is used between 1:50 and 1:10 using glucose or sucrose, being obtained the best results with the last relationship.

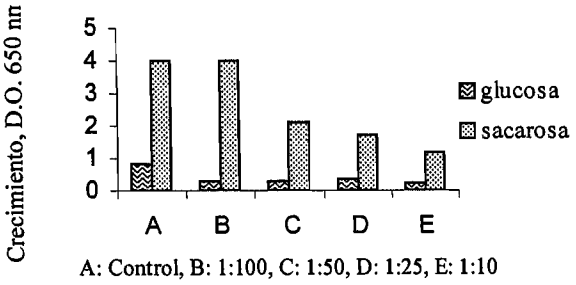

Fig. 1. Inhibitory effect of broths of Streptomyces MC2 about the growth of the *L. mesenteroides 1101*-1.

Study of inhibition of the super of *B. theobromae* 715 with concentrations of jasmonic acid on Leuconostoc.

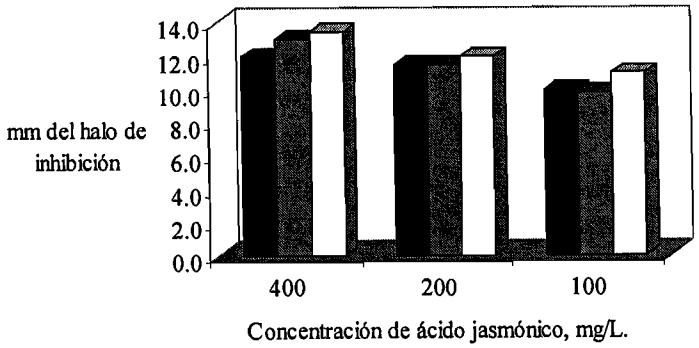

Fig.2. Inhibitory effect of broths of *B. theobromae* 715 with AJ on the *L. mesenteroides* 1101-1, 1-2 and 1-3 (in that order)

It was determined that the super of *B. theobromae* 715 with concentrations of AJ of 600 mg/L inhibited the growth of *L. mesenteroides* L1101-1, 1-2 and 1-3 from the hour 24 at 72, showing the biggest inhibition halo (13 mm) to the 48 h. Fig. 2 shows the inhibitory effect of three concentrations of AJ about the growth of three strains of *L. mesenteroides*.

Although the biggest inhibitions were observed to 400 mg/L of concentration of AJ in the growth medium of the *Leuconostoc*, inhibitory effect was determined with 100 mg/L without remarkable differences. Leuconostoc growth inhibition was observed to higher concentrations to 10 mg/l as can observes in Fig. 3.

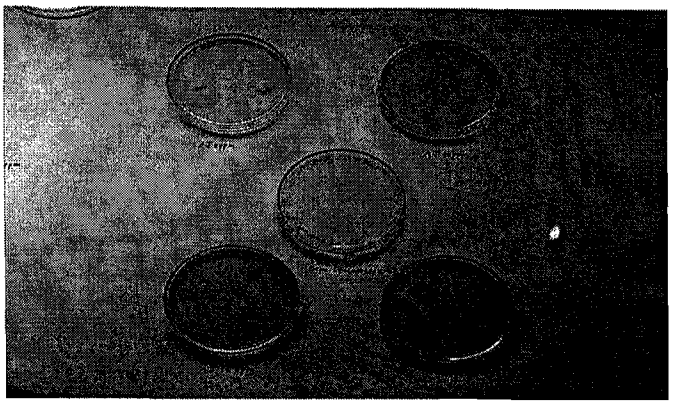

Fig.3. Inhibitory effect of broths of *B. theobromae* 715 with AJ on the *L. mesenteroides* 1101-1 in solid medium.

Add the AJ on the liquid medium of Leuconostoc growth indicated reductions in the consumption of sucrose of 10-20 g/L with relationship to the control and of formation of dextran of 2-6 g/L for respective concentrations of 100 - 400 mg/L of AJ in the medium.

Inhibition effect of other products to laboratory level and bank.

Fig. 4 shows the action of the gluticid, the application of ozone and the inhibitor S about the growth of the Leuconostoc at level pilot and 30 °C. In relation with gluticid can be observed in solid form to two concentrations assays favoured the comparative growth with a control. This can be due to the effect of the ammonium sulfate on which is supported the metabolites of the gluticid and that it has been reported it has a favourable incidence on the metabolism of the Leuconostoc. On the other hand, as much the ozone as the inhibitor S showed a marked character biocide when diminishing in more than two orders the count of Leuconostoc, meeting the hihger inhibition with the application to 0.2% of the inhibitor S. Equally, character fungicide is manifested when reducing in 3 orders the eukaryotes number in the juice. Under the studied conditions a dose of 0.2% of the inhibitor S produces a severe effect microbial to 30 °C, frequent temperature during the period of harvest in the industrial process of the Cuban geniuses.

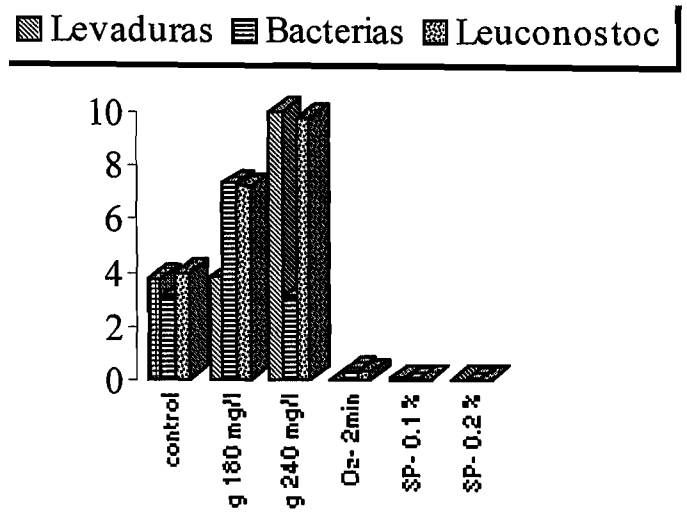

Fig. 4. Inhibitory effect of the Gluticid, the ozone and the inhibitor S on the L. mesenteroides 1101-1 in liquid medium.

Effect of the addition of the inhibitor s in the station of mills of the CAI "España Repúblicana"

According to results it was to carry out the study in the industry with the application of the product.
To know the sanitization state and hygiene of the sugar mill one was made a diagnosis of the situation microbiological of the area of the mills. The Table 2 shows the result of the analysis of the microbial flora in the process of extraction of the juices of the cane.

Table 2. Microbial count of the station of mills of the CAI "España Repúblicana."

	Bacterias mesofilas, UFC/ml	Bacteria producers of polysaccharides, UFC/ml	Yeasts, UFC/ml
Cane juice in the tipper	1.10^6	4.10^7	$1.1.\ 10^7$
Primary juice	$3.\ 10^7$	2.10^7	$1.\ 10^7$
Blended juice	$7.\ 10^6$	$1{,}2.10^7$	$8{,}3.\ 10^6$

Although reports of ranks of 10^7 exist in Cuban blended juices, usually the quantity of bacteria mesofilas and gummy it is usually with exponent between 7 and 8. If is compared these to results with previous reported in Argentina, can be observed that they are considerably smaller those of the Cuban factory to the inferior being in 2 orders to their similar one Argentinean.
These results reflect the little time of demurrage of the cane after having cut in the sugar

mill, indicating a poor deterioration of the vegetable and some hygienic appropriate conditions - sanitary of the station of mills of the CAI España Repúblicana, where they have also been carried out technical modifications to shorten the time of residence of the juice in this stage.

In any way it is beneficial for the factory to adopt a system of control microbiologic that allows to detect problems like:

- Deterioration post campaign of the cane for delay in the production
- Microbiologic situation of the juices in the station of mills for state hygienic-sanitarian of the equipment in this stage

And even:

- Affectation of the quality microbiologic of the sugar and the incidence of the environmental conditions on this.

With a view to determining the effect of the product that excessively has the characteristics of their easy manipulation, as disinfection agent in the area of mills, to reduce the number of micro organisms whose presence is harmful in the industrial process and in the end product.
Taking into account that a concentration of the inhibitor S to 0.2% is high to usually be an employee in the current of bigger flow of the plant like it is the blended juice, where it is necessary to reconcile the cost of the germicide with the sugar that will get lost for microbial action, the effect of inferior dose it was assayed, determining the effect microbial on the total microbial load, bacteria producers of polysaccharides starting from sucrose (Leuconostoc and Bacillus mainly) and yeasts of the primary and blended juice.
The obtained results for concentrations of 0.007% are presented in the Table 3.

Table 3. Effect microbial of the inhibitor S 0.007% on the microbial flora of the primary and blended juice of the CAI "España Repúblicana."

	Bacterias mesofilas, UFC/ml	Polysaccharides producers, UFC/ml	Yeasts, UFC / ml
Primary juice			
0	$3.\ 10^7$	$2.\ 10^7$	1.10^7
1er ttmto			
1 min	$1{,}5.\ 10^6$	$2{,}7.\ 10^6$	$5.\ 10^5$
5 min	$8{,}9.\ 10^5$	$1{,}2.\ 10^6$	$3{,}9.\ 10^5$
2do. Ttmto	$1.\ 10^5$	$6{,}1.\ 10^5$	$2{,}4.\ 10^5$
5 min	$1{,}1.\ 10^5$	$2{,}9.\ 10^5$	$4{,}0.\ 10^5$
30 min	99,6	98,5	96,0
% inhibition	$7.\ 10^6$	$1{,}2.\ 10^7$	$8{,}3.10^6$
Blended juice			
0	$2{,}6.\ 10^5$	$3{,}8.\ 10^6$	$6{,}2.\ 10^5$
1er ttmto	$9{,}3.\ 10^5$	$1{,}3.\ 10^6$	$1{,}4.\ 10^6$
1 min	$7{,}5.\ 10^5$	$3{,}2.\ 10^5$	$1.\ 10^6$
5 min	$4{,}5.\ 10^5$	$4{,}9.\ 10^5$	$7{,}3.\ 10^5$
2do. Ttmto	93,6	95,9	94,2
5 min			
30 min			
% inhibition			

In this study, times of contact were assayed during a maximum period of 30 min. The obtained data allow to conclude that dose of 0.007% with 30 contact min is effective to inhibit above 90% and until the bacteria initial population 99,6% and above 96% of the micro organisms producing of polysaccharides and able to degrade sucrose, as well as above 94% of the present yeasts.
The results indicated that still with 1 min. of contact the product is able to exercise effect immediate microbial to its addition, what is manifested by the abrupt reduction and the high inhibition of the growth of the three microorganism groups.

Effect of the addition of the inhibitor S on the purity of the clarified juice

The inhibitor S was applied to two concentrations looking for effect stabilizer in the purity of the juice. In the sample witness (Table 4) a decrease took place from the purity of 4.8 units to the 12 hours, while with the addition of the inhibitor s an inferior decrease took place to the 2 units and the stabilization of the juice took place almost completely in the variant 3. The juice tried with biocide retained its dark brown colour, fresh scent, pH without big variations to the initial. On the contrary and with a strong contrast, the juice non treaty turned clear brown, with a strong scent to alcohol and pH and purity markedly smaller. The stabilisation effect has not been reported for other agents of commonly used sanitization in the sugar industry.

Table 4. Effect of the addition of the inhibitor S on the purity of clarified juice.

	purity	pH	Bacterias
v1. witness	81.56	4.53	1.1e6
v2. 0.003%	84.99	4.83	Lower to 10e2
v3. 0.006%	86.09	4.94	Lower to 10e2

Behaviour of the stability of the blended juice (strain CO 65 - 357) with the addition of the inhibitor S.

In the table 5 the results of the application of the inhibitor S are shown (0.005%) on the blended juice of the cane (strain CO 65 - 357) in Tucumán, Argentina.

Table 5. Behaviour of the stability of the blended juice (strain CO 65 - 357) with the addition of The inhibitor s (0.005%).

	Hour 0		Hour 24	
	Witness	Treated	Witness	Treated
Brix	15.13	14.88	14.83	15.08
Pol	13.66	13.4	13.58	13.42
ARL (%)	0.24	0.23	1.00	0.16
Polysaccharides, ppm	2459	2491	16 519	2961

According to these results decreases of 6 times were observed with relationship to the witness non treaty in free reducers and polysaccharides that which indicates decrease in the dextrans formed and therefore in the liberated fructose. This result has a sensitive importance economic when diminishing the losses of sugar for formation of polysaccharides in the detained cane or during the stops of the industrial process.

CONCLUSIONS

1. Super obtained from Actinomyces and B. theobromae has inhibitory effect on the L. mesenteroides. Same this effect was observed with the application of ozone and the inhibitor S, being observed the biggest effects with the addition of the last one.
2. Dose of 0.007% of the inhibitor S with 30 min. of contact it was effective to inhibit above 90% and until the bacteria initial population 99,6% and above 96% of the microorganisms producing of polysaccharides and able to degrade sucrose, as well as above 94% of the present yeasts in the primary and blended juice.
3. The inhibitor S is able to stabilize the present sucrose in the juices, besides its effect microbial, with that which you cannot compare with none of the disinfection products that commercially are added in the mills.

In summary, the inhibitor S can be used indeed as agent sanitizante for the station of mills of the sugar geniuses, inhibiting the growth of micro organisms and stabilizing the present sucrose in the juices.

The inhibitor S is innocuous for the man and it does not affect the quality of the sugar final, because its content in metallic salts is to low concentrations in relation with the volume where it is applied and several hundreds of times inferior to the limits allowed in a human food and in the own sugar. On the other hand, the jasmonic acid has carried out by CENPALAB the following biological assay: oral sharp toxicity, dermal sharp toxicity, ocular irritation in vitro and in alive, mutagenesis on the micronucleus of the bone marrow, mutagenesis on the head of the sperm and cytogenesis. All the assays were innocuous for the man, that which demonstrates the possibility of the use of the product without risks for the man health and for the environment.

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Isolation and cloning the genes involve of polysaccharides production in *Leuconostoc*

The utilized strains for this work were the following ones:

For the Cuban part:

B/110-1-1

B/110-1-2

B/110-1-3

C-2

For the Argentinean part:

T6

G2

G5

8293

For the Mexican part:

B512 - control

CW28

IBTPQ

LEV.S

1. Dextransucrase production

The chart 1 sample the enzymatic activities taken place by each one of the strain:

Code of the strain	Activity in super, U/mL	Activity in the Pellet, U/mL (in 5 buffer mL)
B/110-1-1	1,68	12,98
B/110-1-2	1,69	11,02
B/110-1-3	1,48	1,73
C-2	1,16	5,06
T6	0,81	13,09
G2	0,85	11,10

G5 8293 B512 - control CW28	2,06 0,60	9,29 12,59 0,22 1,65

As it can be observed they found differences as for the enzymatic production of the strain and to the soluble activity in the super and associated to cells. The activity of the enzyme varied from 0.6 up to 2.06 U/mL.

2. **Characterization of the dextransucrase**

- Determination of the molecular mass to know the sizes of the produced enzymes, using the electrophoresis method in gel (SDS-PAGE) coupled to the determination of the enzymatic activity to detect the activity of the active bands.

Cuban strains: Super of the B/110-1-1, B/110-1-2, B/110-1-3
Gel of proteins: dextransucrase with two bands: 240 and 190 kDa
Activity gel: the band of 240 no express activity in 110-1-1 1-2. The band of 190 kDa if it presents activity dextransucrase in the three strains

The activity gel underwent treatment with dextranase and the activity bands disappeared what confirms the presence of enzyme dextransucrase and therefore the dextrans formation.

Analyzing the pellet of activity of those strains:
Two bands appear in the three strains with a weight of 200 kDa and three of 240 kDa. The band of 240 no express activity in 110-1-3

Strains C2, 8293 and T6 (in that order):
The C2 has a dextransucrase and two fructosiltransferases, the 8293 have two dextransucrases, and T6 has a dextransucrase and a fructosiltransferase.
The dextransucrases of the three strains corresponds to the 220 and 190 kDa. The fructosiltransferses is of 150 kDa.
When trying with dextranase the activity gels the dextransucrase they disappear and the fructosiltransferase bands are observed. A treatment was applied with inulinase and the bands corresponding to fructosiltransferese disappeared in this treatment for that that although it is something that should be checked in other rehearsals, it seems to be that the stump C2 produces inulinase of high molecular weight and levan, that which application being could in the prebiotics production and very little reported in the literature starting from Leuconostoc.

Argentinean strain: G2, T6 and 8293

The G2 presents an active dextransucrase of 240 kDa, in the T6 two dextransucrase and a fructosiltransferase appear and the 8293 present three active dextransucrase.

On the other hand, the G5 has two dextransucrase: one of 180 and one of 150 kDa.

CONCLUTIONS:

- In all the strain active bands were detected, due to the presence of different enzymes (dextransucrase, fructosiltransferase, possibly inulinase) or to the degradation of a single enzyme.

Preliminary economical study of the results of application of inhibitor S.

According to the analysis carried out by International Organisms and other institutions the affectation that takes place in the economic losses for the microbial action it can be exemplified with the following data:

Valuation of the losses for effect of the polysaccharides (data of GEPLACEA, 1987 in a campaign of 5 millions)

4. Losses of 24 000 t of sugar refine and 11 000 t of raw sugar.
5. Increase of 2 800 t of petroleum in the refinement
6. Affectation of sugar quality. Tax for dextran content from 1.5 to 3.5 USD/t

Economic considerations

1. A stop of a typical clarifier of 300 m^3 (existent in the Cuban sugar mills) during 3 hours it usually diminishes the sugar purity in 3 units, that produces a loss of sugar of 2T (670 USD approx.). Bigger stops cause the complete loss of the clarifier, that could be 45 T of sugar (11 727 USD).
2. For the treatment of a clarifier would be needed according to the doses of this study 15 litres of the product with a cost of 0.80 USD/l, for a total of 12 USD.
3. The balance cost - benefit gives highly favourable, allowing an attractive use of the product to avoid the decomposition of the cane juice in the clarifiers during the non operation time of the mill.

The application of the product in the sugar mill during the harvest has the biggest extension possibilities toward other sugar mills of the sugar countries and generalization in the whole country and other countries of the region that confront similar situation during the campaigns of production of sugar. To avoid the decomposition of the juice for the application of a product- which it has not high price and it is easy to add - it guarantees the preservation of the stability of the cane juice with the consequent economic benefit in avoiding losses of sugar valued in the dozens of thousands of freely convertible foreign currency.

On the other hand, this product can be applied in the station of mills of the sugar mill to contribute to the microbial inhibition-that as it was demonstrated in this work values reached above 90% of microbial inhibition - and with it to diminish the sucrose losses in juice and to increase the sugar yields.

The decreases of the contents of polysaccharides can be quantified economically in savings of fuel to avoid increases of viscosity in the juices and for bigger energy

consumptions and for decrease in the taxs to the sugar for decrement of their quality for affectations in the formation of the glass and dextrans concentration that also invalidate its uses in the food industry.

IV. Impact of the achieved result

For diminishing the effect of the micro organisms, the cleaning of the area of mills is usually practiced and later germicide use. At the moment, diverse chemical substances are employees as germicides and considered as disinfection agents for sugar geniuses. Among them they are the halogens, compound organosulfurades and quaternary ammonium product, but they have not been reports that stabilize the present sucrose in the cane juices.

The inhibitor S has superior effects to the disinfection products that commercially are added in the mills, for this reason the present work constitutes a CONTRIBUTION TO THE KNOWLEDGE inside the thematic of the biocides, demonstrated by the result of the search of patent purity.

Inside the described result, they are outstanding contributions as:

5. It was defined for the first time as a result of investigation works a product with the help of jasmonates with disinfectant and stabilizaters properties of the sucrose in juice.
5. It was defined an outline of application of the product in the process of production of the sugar with highly satisfactory results on the microbial inhibition and the preservation of the cane juices.
5. They were carried out industrial demonstrations of application of the product: one in Argentina and another in a Cuban sugar mill. Both results were satisfactory endorsed by the address of the Institutions where it was demonstrated the inhibitory effects of the microbial growth of the product and the conservation of the characteristics of the treated juice-very superior to a witness non treaty - being evidenced the formation of dextrans contents six inferior times in relation with the witness non treaty.

The accreditations related with this document are those that next are related:

Industrial introduction accreditation of the result.

1. Accreditation of the result by the ICIDCA scientific organ
2. Accreditation of the result for the CAI "España Repúblicana"
3. Accreditation of the result for the EEAOC, Tucumán, Argentina

Accreditation of contribution to the knowledge.

Possibilities to patent. "Biocida compuesto por una sal de jasmonato y otros aditivos de uso en la industria azucarera" Application No. : 2004-0213

Mark application: EVIPOL, Application No. 2004-0470, date: 19-07-2004.

V. Dissemination activities

a) Web site

The information for web page of the ISPLI Project have been given and hosted by the web site www.icidca.cu

b) Publications

- Inhibición por métodos biológicos de *Leuconostoc mesenteroides* en la industria azucarera. G. Cerutti, G. Michelena, A. Martínez, K. Diez, E. Carrera. *Sobre los derivados*, Vol. XXXVI, No. 1, 2003
- Aislamiento y caracterización de nuevas cepas productoras de dextranas procedentes de la industria azucarera cubana. G. Michelena, G. Cerutti, A. Martínez, K. Diez, E. Carrera. *Revista Industrial y Agrícola de Tucumán*, in press
- Efecto de cepas de *Actinomycetes* sobre cepas de *Leuconostoc* procedentes de la industria azucarera, in press
- Dextran and dextran derivatives. Monography. G. Michelena, A. Bell, A. Martínez and E. Carrera
- Michelena G.,Martinez A., Cerutti G., Bell A. y col. "Study of the inhibitory effect on the Leuconostoc and other bacteria" International Sugar Journal. Accepted, May 2005

c) Events

a) Inhibición por métodos biológicos de cepas de *Leuconostoc mesenteroides* aisladas en la industria azucarera. G. Michelena, G. Cerutti, A. Martínez, A. Bell, E. Carrera, S. Legrá. X Congreso Internacional de Biotecnología y Bioingeniería. Puerto Vallarta, Jalisco, México

b) Efecto de diferentes métodos de sanitización del jugo de los molinos en un central azucarero. G. Michelena, A. Martínez, A. Bell, E. Carrera, R. Sotolongo, M. Carbonero, S. Armenteros, S. Legrá. XV Forum Nacional de Ciencia y Técnica. Nivel Municipal. Julio 2003. This work was selected Outstanding Work in the XV Forum of Science and Technique, Cuba.

c) VIII International Congress on Sugar and sugar Cane By- products (Diversification 2004)

ISPLI Project was in this international congress in Havana in June 2004 with 3 oral presentations from some results of the project and the brazilian contribution.

d) REDBIO 2004

Dra. Michelena presented the conference" Biotechnology like alternative for the sugar diversification" in the Symposium V" Industrial Biotechnology, Bioprocess and Bioengineery on Tuesday 22 and it had the participation of more than 50 assistants and the intervention of around 12 interested asked on some topic of that exposed. It stood out the interest of Dominican Republic given in the Dr. Daniel Durán of INDOTEC

and of the Dr. Hichez Frias of CIBIO manifested interest of collaboration in the field of the derivates. Also, Dra. Suzelei of Castro of the UNAERP in Sao Paulo, Brazil thanked the collaboration toasted by the ICIDCA in the mark of the project FAPESP in the jasmonic topic.
On the other hand, Dr. Bell, exposed in the shop III of Biocontrol, the same day 22 in the afternoon, Dr. Odair Pereira of Brazil and the Dr. Moises from Argentina that it expressed to congratulation for the results achieved and they expressed their desire that the ISPLI working style would be imitated. Dr. Moises is an outstanding investigator in Agricultural Biotechnology and he was the winner of the medal REDBIO2004.

ISPLI project received the invitation from participating in regional projects and on Thursday 25 in the afternoon the encounter it was made between investigators from Latin America and the Caribbean in an encounter coordinated by Hichez Frías and Jehová Roca, where the Dra. Michelena presented the thematic of investigation of the Project and a wallet of products the logical mark of two regional projects were written:

1. Aggregation of value on sugar cane for small producers.
2. Energetic use of the sugar cane.

These projects will be presented by the National Director of Project Strategies and Sugar Politics of the Sugar Institute of the Dominican Republic (INAZUCAR) to the Ministry of the Agriculture of the Dominican Republic for their approval.

Havana, January 30th, 2005

IV. Anexs

AVAL DEL CONSEJO CIENTÍFICO DEL ICIDCA

En el trabajo "EFECTO DE DIFERENTES MÉTODOS DE DESINFECCIÓN Y ESTABILIZACIÓN DEL JUGO DE CAÑA EN UN CENTRAL AZUCARERO", de la Dra. Georgina Michelena, la MSc. Aidín Martínez y otros autores, se presenta el efecto de diferentes métodos de desinfección y estabilización sobre los jugos de la caña, en la disminución del tenor microbiano y los índices de pureza, lo cual incide directamente en un mayor rendimiento de azúcar.

Se comprobó el efecto biocida del producto Sucarplus a 0,007% sobre la flora microbiana del jugo primario y mezclado. El jugo tratado retuvo su color carmelita oscuro, olor fresco y pH sin grandes variaciones al inicial. Este efecto estabilizante no ha sido reportado para otros agentes comúnmente usados en la industria azucarera.

Igualmente, se demostró la utilización del Sucarplus para la estabilización del jugo de caña mezclado, pues se observaron disminuciones de 6 veces con relación al testigo no tratado en reductores libres y polisacáridos, lo que indica disminución en las dextranas formadas y por tanto en la fructosa liberada. La aplicación del producto en un clarificador durante una parada larga imprevista del ingenio, para evitar las inversiones del jugo, conlleva ahorros económicos valorados en la decena de miles de USD por lo que el balance costo- beneficio es altamente favorable.

Y para que así conste, se expide el presente AVAL en la Ciudad de La Habana a los 14 días del mes de julio de 2003.

Lic. Addiss Bermello Crespo
Secretaria Científica

Dr. Luis O. Gálvez Taupier
Presidente

Blanca 804 y Carretera Central. Ciudad de La Habana. Cuba. Telef:986501 986502 557428. Fax: (537) 338236. E.mail: direccion@icidca.edu.cu. www.icidca.co

CAI España Repúblicana

Perico, Matanzas, Cuba

Perico, 7 de julio de 2003
"Año de Gloriosos Aniversarios de Martí y el Moncada"

A quien corresponda:

Por este medio certificamos que a finales de la zafra 2003 fue realizado en el CAI España Repúblicana la introducción del producto SUCARPLUS para su aplicación en la desinfección del área de molinos por el ICIDCA, lográndose inhibiciones de la población de bacterias y levaduras, pero especialmente de los microorganismos productores de polisacáridos en niveles por encima al 90 %.

Por otra parte, se realizó un ensayo del efecto del producto sobre la estabilidad del jugo clarificado, encontrándose a las 12 horas efectos de conservación del color, el olor y el pH con relación a un testigo no tratado.

El efecto estabilizante del jugo tiene una sensible importancia económica al evitar las pérdidas de grandes volúmenes del dulce durante las paradas del ingenio que pueden invertirse por la acción de microorganismos y no hacerlo factible para la continuación del proceso de fabricación.

Director del CAI España Repúblicana

ESTACION EXPERIMENTAL AGROINDUSTRIAL
OBISPO COLOMBRES (EEAOC)

Av. William Cross 3150 – Las Talitas
4101, Tucumán. Argentina

San Miguel de Tucumán, Argentina, 13 de junio de 2003

A quien corresponda:

Por la presente tengo el agrado de avalar los resultados obtenidos según ensayo realizado con el inhibidor Sucarplus (obtenido en el Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar, ICIDCA) sobre jugo mezclado obtenido de caña de azúcar, cepa CP65-357 cultivada en Tucumán, Argentina, a concentraciones de 0.006 % donde se logró evitar el deterioro del jugo a las 24 horas evidenciado por la disminución de 6 veces la formación de azúcares reductores libres e igual número de veces la formación de dextranas, comparado con un testigo no tratado.

Este resultado es de una incuestionable importancia económica, pues abre las posibilidades de desarrollo de un producto comercial con perspectivas de uso industrial en los ingenios azucareros donde la formación de dextranas y las pérdidas de azúcar por este concepto constituyen un serio problema.

Ing. Geronimo Cárdenas
Director Asistente en
Investigación y Tecnología Industrial

Graciela S. Cuenca
DOCTOR EN BIOQUÍMICA

OFICINA CUBANA DE LA PROPIEDAD INDUSTRIAL

Ciudad de la Habana, 27 de mayo de 2003

INFORME DEL RESULTADO DE LA BUSQUEDA

Datos del Solicitante: Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar
Vía Blanca No. 804, esquina Carretera Central, San Miguel del Padrón, Ciudad Habana

Referencia: Investigación de patentes para determinar la infracción de derechos de Invenciones en Cuba.
Título. **Biocida compuesto por jasmonato de cobre y otros aditivos de uso en la industria azucarera**

CAMPOS DE BUSQUEDA

POR CLASIFICACION INTERNACIONAL DE PATENTES: **A01N**

Para dar respuesta a la solicitud de búsqueda de infracción de derechos en Cuba, hemos realizado la búsqueda en el Fondo Nacional con que cuenta la Oficina que comprende todas las patentes de invención solicitadas en nuestro país, tanto las que se encuentran concluidas como las que están en fase de tramitación hasta el 26 de mayo de 2003. Realizamos la búsqueda basada en los campos de búsqueda señalados, no detectándose ningún documento relacionado con el tema.

No obstante, se hace necesario tener en consideración las solicitudes internacionales presentadas en virtud del Tratado de Cooperación en Materia de Patentes (PCT) referentes al tema. Es necesario mantener una vigilancia sistemática en relación con la existencia de alguna solicitud internacional vía PCT sobre el tema, que haya solicitado los derechos de protección, desde el mismo momento de la solicitud internacional, siempre que se haya designado o elegido a Cuba para su entrada en fase nacional a los 30 meses a partir de la fecha de prioridad invocada y se concedan los derechos correspondientes.

Sin más,

Lic. Yessika Comesaña Perdomo
Examinadora de patentes

Vto. Bueno. Ing. Eva María Pérez
J'Dpto. Invenciones y Modelos Industriales

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CU

OFICINA CUBANA DE LA PROPIEDAD INDUSTRIAL

Para uso exclusivo de la Oficina

REPÚBLICA DE CUBA

CUENTA BANCARIA
MN: 40286110471019
MLC: CITMA-Ciencia y Técnica

No. Solicitud: 2004-0203

Fecha Solicitud: 28.09.04

Hora: 10 **Minutos:** 24

Sección I. Título: Biocida compuesto por una sal de jasmonato y otros aditivos de uso en la industria azucarera.

Sección II. Solicitantes. ☐ **Persona Natural** X **Persona Jurídica**
(En caso de ser Persona Jurídica llenar) X **Estatal** ☐ **Empresa Mixta** ☐ **Privada**

*(Apellidos seguido de nombre(s) y No. Carné de Identidad. (En caso de una **persona jurídica**, la designación oficial completa incluyendo su Denominación Social. En caso de estatal cubana el organismo al que está subordinado, cuando proceda). En la dirección incluir el código postal, ciudad, provincia o estado y el país)*

Instituto Cubano de Investigaciones de los derivados de la Caña de Azucar (ICIDCA).

Esta persona es:
☐ también un inventor.

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Organismo y Centro de Trabajo:
Ministerio del Azúcar. Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar (ICIDCA).

Profesión u oficio:
—

(Dirección centro de trabajo, incluir el código postal, ciudad, provincia o estado y país)
Vía Blanca No. 804 esquina Carretera Central, Ciudad de la Habana, Cuba, Código postal 11000.

Nacionalidad o Ciudadanía: cubana

Sexo
F -
M ☐

Sección III. Otros Solicitantes y/o (otros) Inventores. X **Persona Natural** ☐ **Persona Jurídica**
(Si hay otros solicitantes o inventores reproducir en hoja aparte)

*(Apellidos seguido de nombre(s) y No. Carné de Identidad. (En caso de una **persona jurídica**, la designación oficial completa y organismo al que está subordinado, cuando proceda). En la dirección incluir el código postal, ciudad, provincia o estado y el país)*

Michelena Alvarez Georgina
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Esta persona es:
X inventor únicamente
☐ solicitante únicamente
☐ solicitante e inventor

Teléfono: 830-62-65

Fax: -

Email: -

Organismo y Centro de Trabajo:
Ministerio del Azúcar. Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar (ICIDCA).

Profesión u oficio: Ingeniero Químico

(Dirección centro de trabajo, incluir el código postal, ciudad, provincia o estado y país)

Vía Blanca No. 804 esquina Carretera Central, Ciudad de la Habana, Cuba, Código postal 11000.

Nacionalidad o Ciudadanía cubana

Sexo
F X
M

Sección IV. ☐ **Agente Oficial** X **Representante**

Número: —

(Apellidos seguido de nombre(s), No. Carné de identidad y dirección. En la dirección incluir el código postal, ciudad, provincia o estado y el país)

Chateloin Lorenzo Vivian
Vía Blanca No. 804 esq. Carretera Central, Municipio San Miguel del Padrón. Ciudad de la Habana. Cuba. Código Postal: 11000

Teléfono: 906501, 02 ext. 278

Fax: 33-82-36

E-mail: vivian.chateloin@icidca.edu.cu

Firma y Cuño:

Cont...Sección III. Otros Solicitantes y/o (otros) inventores. X **Persona Natural** ☐ **Persona Jurídica**

*(Apellidos seguido de nombre(s) y No. Carné de identidad. (En caso de una **persona jurídica**, la designación oficial completa y organismo al que está subordinado, cuando proceda). En la dirección incluir el código postal, ciudad, provincia o estado y el país)*

Bell García Antonio
C.I. 38011310480
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Organismo y Centro de Trabajo:
Ministerio del Azúcar, Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar (ICIDCA).

Profesión u oficio:
Lic. Químico

(Dirección centro de trabajo, incluir el código postal, ciudad, provincia o estado y país)
Vía Blanca no. 804 esquina carretera Central, Ciudad de la Habana, Cuba. Código postal 11000.

Nacionalidad o Ciudadanía: Cubana

Sexo
F -
M X

Cont. Sección III. Otros Solicitantes y/o (otros) inventores. X **Persona Natural** ☐ **Persona Jurídica**

*(Apellidos seguidos de nombre(s) y No. Carné de identidad. (En caso de una **persona jurídica**, la designación oficial completa y organismo al que está subordinado, cuando proceda). En la dirección incluir el código postal, ciudad, provincia o estado y el país).*

Martínez Sánchez Aidín
C.I. 55071012318
Dirección: San Miguel No. 657 e/ Lucena y Marquéz González, Municipio Centro Habana, Provincia Ciudad de la Habana.
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Organismo y Centro de Trabajo:
Ministerio del Azúcar, Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar (ICIDCA).

Profesión u oficio:
Lic. Microbiología

(Dirección centro de trabajo, incluir el código postal, ciudad, provincia o estado y país)
Vía Blanca no. 804 esquina carretera Central, Ciudad de la Habana, Cuba, Código postal 11000.

Nacionalidad o Ciudadanía: Cubana

Sexo
F X
M -

Cont. Sección III. Otros Solicitantes y/o (otros) inventores. X **Persona Natural** ☐ **Persona Jurídica**

*(Apellidos seguidos de nombre(s) y No. Carné de identidad. (En caso de una **persona jurídica**, la designación oficial completa y organismo al que está subordinado, cuando proceda). En la dirección incluir el código postal, ciudad, provincia o estado y el país).*

Carrera Bocourt Emilia
Dirección: Edificio E-46 apto. 8, Zona 11, Alamar, Municipio Habana del Este, Provincia Ciudad de la Habana.
Código Postal: 12500

Esta persona es:
X inventor únicamente
☐ solicitante únicamente
☐ solicitante e inventor

Teléfono: -

Fax: -

E-mail: -

Organismo y Centro de Trabajo:
Ministerio del Azúcar, Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar (ICIDCA).

Profesión u oficio:
Técnico en Análisis Químico

(Dirección centro de trabajo, incluir el código postal, ciudad, provincia o estado y país)
Vía Blanca no. 804 esquina carretera Central, Ciudad de la Habana, Cuba, Código postal 11000

Nacionalidad o Ciudadanía: Cubana

Sexo
F X
M ☐

Cont...Sección III. Otros Solicitantes y/o (otros) Inventores. X **Persona Natural** ☐ **Persona Jurídica**

*(Apellidos seguido de nombre(s) y No. Carné de Identidad. (En caso de una **persona jurídica**, la designación oficial completa y organismo al que está subordinado, cuando proceda). En la dirección incluir el código postal, ciudad, provincia o estado y el país)*

Inés Cerutti Graciela
C.I. 12413468
Calle Bolivia No. 135, San Miguel de Tucumán, Argentina.
Código Postal 4000

Esta persona es:
X inventor únicamente
☐ solicitante únicamente
☐ solicitante e inventor

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Fax: -

E-mail: cerutti@unt.edu.ar

Organismo y Centro de Trabajo:
Universidad Nacional de Tucumán.

Profesión u oficio:
Dr. Bioquímica

(Dirección centro de trabajo, incluir el código postal, ciudad, provincia o estado y país)
Ayacucho 491-4000. Código Postal 4000. Ciudad Tucumán.

Nacionalidad o Ciudadanía: Argentina

Sexo
F x
M -

Cont. Sección III. Otros Solicitantes y/o (otros) Inventores. X **Persona Natural** ☐ **Persona Jurídica**

*(Apellidos seguidos de nombre(s) y No. Carné de Identidad. (En caso de una **persona jurídica**, la designación oficial completa y organismo al que está subordinado, cuando proceda). En la dirección incluir el código postal, ciudad, provincia o estado y el país).*

López- Murguía Canales Agustín
C.I: LPCNAG51012609H300
Dirección: Nueva Suiza No. 23 Col. 3ª sección de Reforma, Cuernavaca, Morelos. País México.

Esta persona es:
X inventor únicamente
☐ solicitante únicamente
☐ solicitante e inventor

Teléfono: -

Fax: -

agustin@ibt.unam.mx

Organismo y Centro de Trabajo.
UNAM, México

Profesión u oficio.
Ing. Químico

(Dirección centro de trabajo, incluir el código postal, ciudad, provincia o estado y país)

Ave. Universidad No. 2101, Chamilpa, Cuernavaca, Morelos, México, Código Postal 62250.

Nacionalidad o Ciudadanía: Mexicano

Sexo
F -
M X

Cont. Sección III. Otros Solicitantes y/o (otros) Inventores. ☐ **Persona Natural** ☐ **Persona Jurídica**

*(Apellidos seguidos de nombre(s) y No. Carné de Identidad. (En caso de una **persona jurídica**, la designación oficial completa y organismo al que está subordinado, cuando proceda). En la dirección incluir el código postal, ciudad, provincia o estado y el país)*

Olvera Carranza Clarita
Carnét: OLCRCL69121119M900
Dirección: Privada Colorines No. 76, Col. Chamilpa Cuernavaca., Morelos, México.

Esta persona es:
X inventor únicamente
☐ solicitante únicamente
☐ solicitante e inventor

Teléfono: -

Fax: -

E-mail: clarita@ibt.unam.mx

Organismo y Centro de Trabajo:

UNAM, México

Profesión u oficio:
Lic. Microbiología

(Dirección centro de trabajo, incluir el código postal, ciudad, provincia o estado y país)

Ave. Universidad No. 2101, Chamilpa, Cuernavaca, Morelos, México, Código Postal 62250

Nacionalidad o Ciudadanía:
Mexicana

Sexo
F X
M ☐

Sección V. Declaración de Responsabilidad. *(Llenar si procede)*

X Creada en prestación de servicio estatal o en colaboración con un Organismo de la Administración Central del Estado, Empresa, Institución, etc.

☐ Creada en prestación de servicio estatal o en colaboración con un Organismo de la Administración Central del Estado, Empresa, Institución, etc., y estos últimos no han efectuado la solicitud de registro correspondiente.

☐ Creada fuera del marco de las obligaciones laborales del autor.

☐ Creada como resultado del trabajo colectivo entre dos Organismos de la Administración Central del Estado, Instituciones, etc.

☐ Creada dentro del marco de la colaboración económica y científico-técnica entre una entidad cubana y una extranjera

☐ Presentada en virtud del Tratado de Cooperación en Materia de Patentes (PCT), en el cual la OCPI actúa como ☐ oficina designada u ☐ oficina elegida.

Fecha Solicitud Internacional:	No. Solicitud Internacional:	No. Public. Internacional:

(Nombre oficial completo del Organismo, Empresa o Institución tanto nacional como extranjera que forma parte de la colaboración y dirección. En la dirección incluir el código postal, ciudad y país)	**Firma del Representante y cuño de la Entidad:**
	Fecha del Convenio:

Teléfono:	Fax	E-mail:

Sección VI. Reivindicación de Prioridad. *(Si es más de tres (3), reproducir esta sección en hoja aparte)*

☐ Prioridad Convencional			☐ Prioridad de Exposición	
País	**Fecha Presentación**	**No. Solicitud**	**País**	**Fecha Exposición**

Sección VII. Lista de Verificación.

Esta solicitud contiene:			*Esta solicitud, se acompaña además de:*
1. Instancia	4	Hojas	☐ Hoja de cálculo de tarifas
2. Resumen	1	Hojas	☐ Copia Certificada Prioridad Convencional
3. Descripción	6	Hojas	☐ Copia Certificada Prioridad por Exposición
4. Reivindicaciones	2	Hojas	☐ Depósito de Cepa
5. Dibujos	-	Hojas	☐ Condición Sucesor Legal
6. Relación de secuencia de Nucleótidos y Aminoácidos (o Diskette)	_	Hojas	☐ Poder
7. Total	13	Hojas	Otros 2 Hojas de Investigaciones previas

Sección VIII. Tipo de documento de protección que se solicita.

X Certificado de Autor de Invención ☐ Certificado de Patente de Invención
☐ Certificado de Autor de Invención de Adición ☐ Certificado de Patente de Invención de Adición

Sección IX. Firma del Solicitante, Agente Oficial o Representante:

Sección XI. Persona que recepciona la solicitud

(Apellidos y nombre(s))	Firma	Cuño

Para uso exclusivo de la Oficina

CENDA

Centro Nacional de Derecho de Autor

Registro: 721 - 2004

CERTIFICACION DE DEPOSITO LEGAL FACULTATIVO DE OBRAS PROTEGIDAS.

La que suscribe, Lic. Regla Isabel Mejías Rogers, Especialista del Departamento Jurídico del Centro Nacional de Derecho de Autor, **CENDA** deja constancia de que, previa comprobación, ha sido admitida en el área de depósito legal de esta Institución la obra, protegida por la legislación vigente de Derecho de Autor en la República de Cuba cuyos pormenores se describen a continuación:

Título : Dextrana y sus derivados.

Autor /(es): Georgina Lourdes Michelena Alvarez; Antonio Bell García; Aidín Martínez Sánchez; Emilia Carrera Bocourt.

Titular: Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar.

Tipo de Obra: Literaria

Características: Obra que recoge parte de las tareas de investigación realizadas por los autores en cuatro décadas (1960 – 2000) de trabajo vinculadas a explorar el potencial de la dextrana como producto derivado de la sacarosa, con el objetivo de diseñar procesos que diversifiquen la industria azucalera de manera revalorizada y se desarrollen nuevos productos que contribuyan a la industria farmacéutica cubana.

El presente documento que otorga la fe pública del acto de creación. La existencia y la titularidad originaria en esta fecha de la obra descrita, sólo constituiría prueba de primera vista ante cualquier litigio respecto a la autoría y explotación de la misma.

Dado en Ciudad de la Habana, a los 25 días del mes de marzo de 2004.

Funcionario Público

Autor

Para proteger la creación

Calle 15 Nº 604 e/ B y C, Vedado, Ciudad de La Habana, Cuba, 10400. Apartado Postal 4521.
Teléfono: (53-7) 832 3571 – 73 Fax: (53-7) 66 2030 E-mail:
http://www.cenda.cu

LA COMISIÓN MUNICIPAL DEL

FÓRUM DE CIENCIA Y TÉCNICA

DE SAN MIGUEL DEL PADRÓN

otorga el presente

DIPLOMA

A la ponencia

EFECTO DE DIFERENTES MÉTODOS DE DESINFECCIÓN Y ESTABILIZACIÓN DEL JUGO DE CAÑA EN UN CENTRAL AZUCARERO .

Autor (es):

DRA. GEORGINA MICHELENA ÁLVAREZ
MSC. AIDÍN MARTÍNEZ

en su condición de RELEVANTE

EN EL XV FORUM DE CIENCIA Y TECNICA

"... La preservación de la independencia de este país depende fundamentalmente de la ciencia y la técnica..."

Fidel Castro Ruz

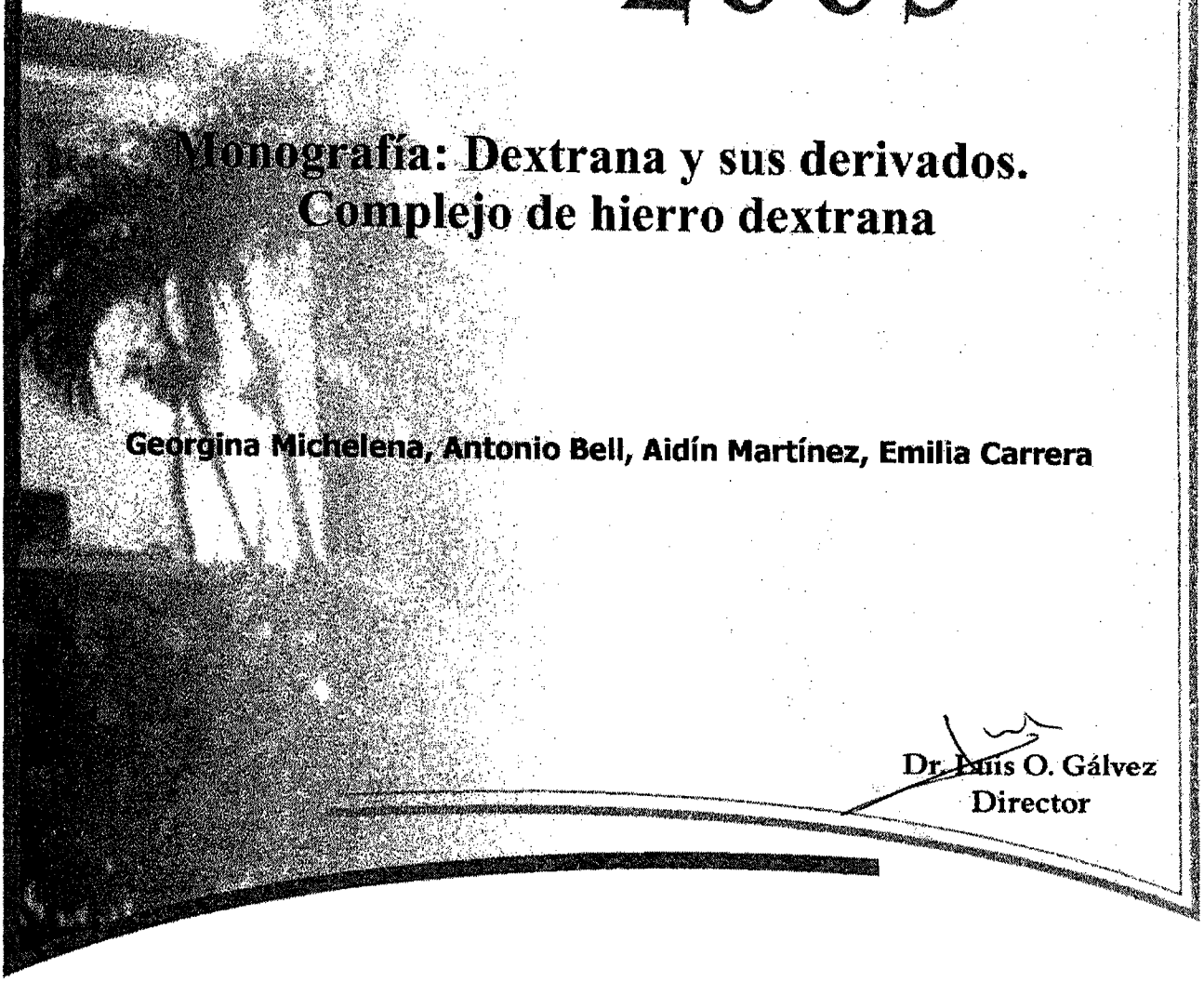
Dado en San Miguel del Padrón a los 26 días del mes de Septiembre de 2003

Luis H. Serrano Pérez
Pte. Comisión Municipal
Fórum de Ciencia y Técnica

ASAMBLEA MUNICIPAL DEL PODER POPULAR
VICEPRESIDENTE
SAN MIGUEL

ICIDCA

logros ICIDCA 2003



Monografía: Dextrana y sus derivados. Complejo de hierro dextrana

Georgina Michelena, Antonio Bell, Aidín Martínez, Emilia Carrera

Dr. Luis O. Gálvez
Director

To whom it may concern:

Reading the Annual Report – 2003 of the Project "Increase in sugar production by microbiological inhibition of Leuconostoc spp. and other bacterias", was a scientific satisfaction and the opportunity to know about a real contribution to the cane sugar juice at the milling station is one of the most important concerns in looking for an industrial efficiency of the sugar mill.

The results of such deterioration drive not only to a loose of sucrose and consequently a very well known reduction of the factory yields, but also is the cause of the formation of non-sucrose products, mainly polysaccharides like dextran, that increase the viscosity of the factory's intermediate materials, depleting the effectiveness of the crystallization process and consequently the overall recovery of the main product of the sugar mill.

To develop biological ways and means to overcome such adversity by the inhibition of bacterial growth avoiding the use of unhealthy chemicals, is not only economically convenient but also a real contribution to ensure a cane sugar production environmental friendly that render a more ecological sugar, an attractive product with a certain niche in the world sugar market.

That reasons obviously drive me to advise the convenience to support an extension of the Project to go deeply in this attractive technological approach.

Dr. Oscar Almazán, Ph.D.
Honorary Life Member of the International Society of Sugar Cane Technologist
Doctor in Biological Science
Doctor "Honoris Causa" in Technological Science
Senior Adviser to the Cuban Ministry of Sugar

Havana, February 27, 2004