

Productividad Académica de la LGAC de Biotecnología Microbiana

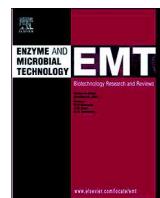
Las publicaciones de los PTC de la LGAC de Biotecnología Microbiana, en comparación con el periodo anterior, se incrementaron en este periodo de evaluación y se publicaron en revistas de mejor impacto, mientras se han enfocado en tres temáticas básicas: *i)* fermentación alcohólica de agave y aprovechamiento de las levaduras asociadas, *ii)* valorización de subproductos e insumos de bajo valor comercial y, *iii)* biorremediación de suelos. Así mismo, se ha colaborado con grupos de investigación de otras instituciones, en investigaciones relacionadas con las temáticas ya mencionadas.

La labor científica de la LGAC se complementa con la colaboración con grupos de investigación de otras instituciones, tanto nacionales como extranjeras, en donde se han generado un total de 13 artículos de investigación en colaboración con estudiantes en el periodo, de los cuales 12, son con estudiantes de doctorado

PRODUCCIÓN ASOCIADA A LOS ESTUDIANTES-PTC MIEMBROS DE LA LGAC BIOTECNOLOGIA MICROBIANA.

BIOTECNOLOGIA MICROBIANA	
1	Vazquez-Ortega, P. G., Alcaraz-Fructuoso, M. T., Rojas-Contreras, J. A., López-Miranda, J., & Fernandez-Lafuente, R. (2018). Stabilization of dimeric β -glucosidase from <i>Aspergillus niger</i> via glutaraldehyde immobilization under different conditions. Enzyme and microbial technology 110, 38-45.
2	Sánchez-Castañeda, A. K., Athès, V., Moussa, M., López-Miranda, J., Páez-Lerma, J. B., Soto-Cruz, N. O., & Trelea, I. C. (2018). Modeling of isoamyl acetate production by fermentation with <i>Pichia fermentans</i> in an aerated system coupled to in situ extraction. Process Biochemistry.65, 11-20
3	Bailón-Salas, A. M., Ordaz-Díaz, L. A., Valle-Cervantes, S., Lopez-Miranda, J., Urtiz-Estrada, N., Páez-Lerma, J. B., ... & Rojas-Contreras, J. A. (2017). Bacterial Diversity in Two Aerated Lagoons of a Pulp and Paper Effluent and their Interaction with a Commercial Inoculum using PCR-DGGE. BioResources, 12(3), 5487-5501.
4	Cisneros de la Cueva S., Hernández Rodríguez C., Soto Cruz, N.O., Rojas-Contreras, J.A., López- Miranda, J. (2016) Changes in Bacterial Populations During Bioremediation of Soil Contaminated with Petroleum Hydrocarbons Water, Air, & Soil Pollution, Vol.227, Pag.1-12.
5	Cisneros-de la Cueva, S., Martínez-Prado M.A., López-Miranda, J., Rojas-Contreras, J.A. and H. Medrano-Roldán H. (2016) Aerobic degradation of diesel by a pure culture of Aspergillus terreus kp862582 , Revista Mexicana de Ingeniería Química, Vol.15, Pag.347-360.
6	Nuñez-Guerrero M.E., Páez-Lerma J.B., Rutiaga-Quiñones O.M., González-Herrera S.M., Soto-Cruz O.N. (2016). Performance of mixtures of <i>Saccharomyces</i> and non- <i>Saccharomyces</i> native yeasts during alcoholic fermentation of Agave duranguensis juice. Food Microbiology. 54, 91-97.
7	De los Rios-Deras, G.C., Rutiaga-Quiñones, O.M., López-Miranda, J., Páez Lerma, J., López, M.G. and Soto-Cruz, N.O (2015). Improving Agave durangensis must for enhanced fermentation , Revista Mexicana de Ingeniería Química, Vol.14, Pag.363-371.
8	Alma D. Orozco-Cortés, Gerardo Álvarez-Manilla, Gerardo Gutierrez-Sánchez, Olga M. RutiagaQuiñones, Javier López-Miranda and Nicolás O. Soto-Cruz. (2015) Characterization of fructans from <i>Agave duranguensis</i> . African Journal of plant Science. 9(9), pp. 360-367
9	Rodríguez-Sifuentes, L., Páez-Lerma, J.B., Rutiaga-Quiñones, O.M., Ruíz-Baca, E., Rojas-Contreras, J.A., Gutiérrez-Sánchez, G., Barrio, E. and Soto-Cruz, N.O. (2014). Identification of a yeast strain as a potential stuck wine fermentation restarter: a kinetic characterization. CyTA Journal of Food., Vol.12, Pag.1-8.
10	Ordaz-Díaz L.A., Rojas-Contreras J.A., Rutiaga-Quiñones O.M., Moreno-Jiménez M.R., Alatriste-Mondragón F. and Valle-Cervantes S. (2014). Microorganism degradation efficiency in bod analysis formulating a specific microbial consortium in a pulp and paper mill effluent. Bioresources. 9. 7189-7197.
11	Hernández-Carbajal, G.R., Rutiaga-Quiñones, O.M., Pérez-Silva, A., Saucedo-Castañeda, G., Medeiros, A.B.P., Soccol, C.R, and Soto-Cruz, N.O. (2013). Screening of native yeast from Agave duranguensis spontaneous alcoholic fermentation with potential for production of Isoamyl acetate. (Brazilian Archives of Biology and Technology. Vol.56, Pag.357-363.
12	Páez-Lerma, J. B. Arias-García, A., Rutiaga-Quiñones, O. M., Barrio, E. and Soto-Cruz, N. O. (2013). Yeasts isolated from the alcoholic fermentation of Agave duranguensis during mezcal production, Food Biotechnology, Vol.27. 342-356.
13	Fileto-Pérez H.A., Rutiaga-Quiñones J.G., Aguilar-González C.N., Páez J.B., López J. and Rutiaga-Quiñones O.M. (2013). Evaluation of <i>Eichhornia crassipes</i> as an Alternative Raw Material for Reducing Sugars Production. Bioresources.8 (4) 5340-5348.

cuatro variedades (pinto villa, pinto saltillo, pinto mestizo y flor de mayo). Revista Mexicana de Ingeniería Química,
Vol.10, Pag.17-28.



Stabilization of dimeric β -glucosidase from *Aspergillus niger* via glutaraldehyde immobilization under different conditions



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ARTICLE INFO

Keywords:

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Glutaraldehyde versatility
Enzyme immobilization
Improved enzyme activity upon immobilization
Amino-agarose beads
Glucosidase

ABSTRACT

The dimeric enzyme β -glucosidase from *Aspergillus niger* has been immobilized on different amino-agarose beads at pH 5 and 7, exploiting the versatility of glutaraldehyde. The stability of the free enzyme depended on enzyme concentration. Immobilization via ion exchange improved enzyme stability/activity, depending on the immobilization pH. However, the enzyme was desorbed in 75 mM NaCl at pH 7 and some stability/enzyme concentration dependence still existed.

Treatment of these biocatalysts with glutaraldehyde increased enzyme stability (e.g. at pH 5, after incubation under conditions where the enzyme just ionically exchanged was fully inactivated, the activity of the glutaraldehyde treated enzyme remained unaltered). Immobilization on glutaraldehyde pre-activated supports yielded a higher increase in enzyme activity, but the stabilization was lower. While when measuring the enzyme activity at pH 4 there were no changes after immobilization, all immobilized enzymes were more active than the free enzyme at pH 6 and 7 (2–3 times). The K_i/K_m ratio did not significantly decrease in any immobilized biocatalysts, and in some cases it worsened in a significant way (by a 9 fold factor using preactivated supports). The new biocatalysts are significantly more stable and avoid enzyme subunit desorption, being the immobilization pH a key point in their design.

1. Introduction

The demand for renewable energy sources has moved from biodiesel to bioethanol produced from lignocellulosic materials, the most abundant polymers in nature [1–4]. Lignocellulosic biomass is one of most promising renewable feedstocks for ethanol production, which is a suitable fuel, due to its high octane number, vaporization heat, and compatibility with motor vehicles [5]. The first step in the production of bioethanol is the hydrolysis of cellulose to get glucose. In this regard, the use of enzymes presents some advantages, like the prevention of side-reactions produced using acid catalysis that can hinder the fermentation process [1–4,6].

The enzymatic hydrolysis of cellulose is accomplished from the synergistic action of endoglucanases, exoglucanases I and II and β -glucosidases. These enzymes are found commonly in fungal species (*Penicillium verruculosum*, *Trichoderma reesei*, *Aspergillus niger*, *Sporotrichum thermophile*) [7]. The first enzymatic hydrolytic steps are

performed by endoglucanase and exoglucanase activities, and they produce elevated concentrations of cellobiose (a reducing sugar that is formed by two β glucose molecules linked by a β (1 → 4) bond), which is the substrate of β -glucosidase [8,9]. β -glucosidases may be applied in other processes such as fine chemicals and medicines production [10]. Thus, they are also used to produce the release of some glycosylated substances (like flavors or terpenes in wine and other products [11–18]) or the production of some glycosylic bonds [19–21]. The importance of β -glucosidases in industrial and environmental processes has been summarized in different reviews and investigations [22–24].

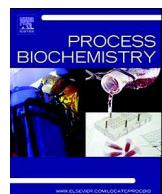
Thus, biocatalysis is a good option to transform cellulose into glucose, but some problems, such as the price and moderate enzyme stability under operational conditions are hindering their industrial implementation [25,26]. One solution to both problems may be enzyme immobilization. If the immobilized enzyme is stable enough, it may be reused, and that will decrease the final price of the biocatalyst [27–30]. A proper immobilization may improve enzyme stability via multipoint

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Modeling of isoamyl acetate production by fermentation with *Pichia fermentans* in an aerated system coupled to *in situ* extraction



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ARTICLE INFO

Chemical compounds studied in this article:

Isoamyl acetate (PubChem CID: 31276)

Decane (PubChem CID: 15600)

L-leucine (PubChem CID: 6106)

Keywords:

Aroma production

Fermentation kinetics

Partition coefficient

Simulation

Natural flavoring substance

ABSTRACT

This study deals with the production of isoamyl acetate (IAA) by fermentation of sugar cane molasses with the strain *Pichia fermentans* ITD00165, using L-leucine as precursor. A mathematical model that describes the experimental data from fermentation was developed for its use as a tool for further process optimization. The fermentation system was constantly aerated and coupled to liquid-liquid *in situ* extraction with decane as the recovery solvent. Thus, the model integrates the biological production of IAA, its partition coefficient in the two liquid phase system and the stripping effect of aeration. A productivity of $26 \text{ mg L}^{-1} \text{ h}^{-1}$ was obtained with addition of 4 g L^{-1} of L-leucine at 12 h of fermentation. The use of the model for process optimization was explored. According to it, the maximum theoretical productivity that can be obtained is $63 \text{ mg L}^{-1} \text{ h}^{-1}$. The model was used to determine that 1.6 g L^{-1} is the minimum concentration of L-leucine that can be added without significantly reducing IAA production. Also, it makes possible to propose an adequate decane/culture medium ratio, to have a desired final concentration and amount of recovered IAA. This value can be adjusted based on the needs of further purification steps and is useful to define a global economic optimum of the process.

1. Introduction

Esters of short-chain fatty acids are important flavor and fragrance compounds widely used in the food and beverage industries. Isoamyl acetate (IAA) is characterized by its strong smell of banana which gives it a very important place in food, pharmaceutical and perfumery industries with a demand of 75 tons per year in USA alone in 2010 [1–3], increasing over the years. This substance is obtained by chemical synthesis, extraction from natural sources, or fermentation [4].

In recent years, the interest towards the production of flavor compounds through white biotechnology processes over traditional methods has increased. Principal reasons are that chemical synthesis often consists in an environmentally unfriendly production process, with important drawbacks such as poor reactions selectivity resulting in racemic mixtures, low yields, and high downstream costs [5,6]. Also, consumers have developed an apprehensive attitude towards these synthetic compounds, especially if the products are related to food or domestic usage. Moreover, the extraction of flavoring compounds from natural sources gives very low yields and has potential difficulties with

obtaining the raw material [3,4,7]. Contrary to chemical synthesis, flavors obtained by fermentation can have the “natural flavoring substance” classification by the European (EC No 1334/2008) and U.S. (21CFR101.22) regulations, which allows using them safely as an additive in food and beverages. This has been the main reason for developing biochemical processes over the years, but presently, after years of research, the inherent advantages of white biotechnology processes are the driving force for its application: operation under mild and more environmentally friendly conditions, as well as chemical and stereo specificity of the obtained compounds, are the most important ones [8].

There are several published studies focused on IAA biological production. They include enzymatic synthesis using lipases and esterases [1,3,9–15] and fermentation process by microorganisms, especially yeasts strains [6,16–20]. Whole cell fermentation can be a more economical method than enzymatic systems and easier to scale up to industrial level. There are some encouraging studies with interesting IAA productivities in the literature; for example, Yilmaztekin et al. [6] used a *Williopsis saturnus* strain in a medium composed of beet molasses and

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Bacterial Diversity in Two Aerated Lagoons of a Pulp and Paper Effluent and their Interaction with a Commercial Inoculum using PCR-DGGE

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Aerated lagoons are a main unit operation for wastewater treatment in the paper industry. Many such operations involve inoculation with bacterial formulations in which *in situ* effectiveness has not been proven; this can be translated into low efficiency in treatment and unnecessary investments. Lack of knowledge of bacterial biodiversity present in a lagoon limits the capacity to exploit the maximum degradation. To overcome such problems, various methods to identify and study these microorganisms have been developed. In this study, a PCR-DGGE analysis was performed to estimate the bacterial diversity and to verify the presence of bacteria present in a commercial inoculum in two aerated lagoons of a pulp and paper effluent. Phylogenetic affiliation of predominant member's correspondent to γ - and β -proteobacteria and Firmicutes were found. The dominant bacteria present in lagoon 2 belonged to the following genus *Microbacterium* sp., *Rhodocyclaceae* sp., *Eubacterium* sp. and *B. subtilis*. In lagoon 1 the dominant genus included *Microbacterium* sp., *Rhodocyclaceae* sp., *Tepidimonas* sp., *Acetanaerobacterium* sp., and *Flavobacteria* sp. The two characterized lagoons were not similar to the commercial inoculum. In addition, non-dominant bacteria (less relative intensity) were composed mostly of bacteria of the commercial inoculum.

Keywords: *Pulp and paper effluent; Bacterial diversity; PCR-DGGE; 16S rDNA; Commercial inoculum*

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INTRODUCTION

Wastewaters of pulp-and-paper mills can be potentially polluting and very dangerous, so they should be treated before being discharged (Ashrafi *et al.* 2015). The most common aerobic biological methods used in the treatment of pulp mill effluents are aerated lagoons (Bajpai 2012). Of the microorganisms involved in the depuration process, bacteria stand out; they are able to convert organic matter to carbon dioxide, water, and biomass, which can be removed by physical methods (Welander *et al.* 1997; Forster *et al.* 2003). The stability and permanence of bacteria in the system ensures an efficient process. However, lagoons are subjected to various perturbations such as variations in pH, high organic loads, presence of toxic compounds, and seasonal changes (Mueller *et al.* 1977). To counteract these effects, a biomass support material (Welander *et al.* 1997) or lagoons that are inoculated with commercial inoculum are introduced.

Changes in Bacterial Populations During Bioremediation of Soil Contaminated with Petroleum Hydrocarbons

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1 Introducción

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AEROBIC DEGRADATION OF DIESEL RY A PURE CULTURE OF *Aspergillus terreus* KP862582

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Abstract

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Resumen

Este estudio se realizó en un suelo natural para analizar la actividad de un cultivo puro de *Aspergillus terreus* KP862582 en la degradación del aceite diesel. Se realizó una reacción en cadena de la polimerización (PCR) para determinar la actividad de este organismo en la degradación del aceite diesel. Se utilizaron suelos de diferentes tipos y condiciones para evaluar la actividad del organismo. Los resultados mostraron que el organismo es capaz de degradar el aceite diesel en un plazo de 10-10 días, lo que indica que es un organismo prometedor para la degradación del aceite diesel en suelos naturales.

1 Introduction

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* Corresponding author. E-mail: adriana.martinez@orst.edu



Food Microbiology

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Performance of mixtures of *Saccharomyces* and non-*Saccharomyces* native yeasts during alcoholic fermentation of *Agave duranguensis*

Jill!Ce



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Oiga Miriam Rutiaga-Quinones, Silvia Marina González, Her re ra.

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Introduc1ion

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), Selection of the .app1'opnate strJms is generally bi1\$cd on cn1rr1,1 such ,b fermenta1iv<'power. roJeraocc to ethanul. e,h,m& y1('1d. toller phcnotyj)E", ;tnd low acetic acid i:>rnd u<tio n (1).



Revista Mexicana de Ingeniería Química

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PROVING Agave *dillingerensis*. MUST FOR ELIMINANCE FERMENTATION. C/N
RATIO EFFECTS ON LEZCAL COMPOSITION AND SEXSORY PROPERTY IRIS

¡F E .JORA !WJNT O OEL IMOSTO DE A a,-e durmtfiensis PARA LA FER JEN TACJØ N
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MEZCAL Y LAS PROPIE DADES SENSO RI ALES

G.C. De lo Rio--Dcras¹. O.M. Ruu:gi a,Quhbnc'.

J. I. López-Ortega, M. Hernández, J. B. Púcl, J. C. Nuñez, V. Gómez, T. Gómez, L. N. O. Soto-Castaño

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Ful/ Length Research Paper

Characterization of fructans from *Agave durangensis*

Alma D. Orozco-Cortés¹, Gerardo Alvarez-Manilla², Gerardo Gutiérrez-Sánchez², Oiga M. Rutiaga-Quiñones¹, Javier López-Miranda¹ and Nicolás O. Soto-Cruz[•]

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Agave plants are members of the Agavaceae family and utilize crassulacean acid metabolism (CAM) for CO₂ fixation. Fructans are the main photosynthetic products produced by Agave plants, and are the principal source of storage carbohydrates. The aim of this work was to determine the chemical and molecular characterization of fructans from *Agave durangensis*. Fructans were extracted from 10 year old *A. durangensis* plants. Trimethylsilyl derivatization was employed to determine the monomer composition. The linkage types in these carbohydrates were determined by methylation followed by reduction and O-acetylation, and finally analysis by gas chromatography-mass spectrometry (GC-MS). Samples were shown to contain t-JI-D-Fru f, t-a-D-Glup, i-a-D-6,Glup and 1,6-dl-D-Fru linkages. The analysis of the degree of polymerization (DP) was confirmed by MALDI-TOF-MS showing a wide DP ranging from 2 to 29 units. The analyses performed revealed that fructans from *A. durangensis* are formed of 97.11% fructose and 2.89% glucose, and are a complex mixture of fructooligosaccharides of the neo-fructan type containing principally 13(2-1) and 1-(2-6) linkages, with branchmoieties.

Key words: Degree of polymerization(DP) GC-MS MALDI-TOF-MS,

INTRODUCTION

Mexico has been considered the center of origin and biodiversity of the *Agave* genus, due to the taxonomic diversity found within its borders. Of the 310 species reported, about 272 can be found in this country,

Members of the Agavaceae family are distributed throughout Mexico, and are well adapted to both arid and semi-arid regions (García-Mendoza and Gálvez, 1995). They have undergone both morphological and

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Abbreviations: OMSO: Dimethyl sulfoxide; TFA: trifluoroacetic acid, EtOH: ethanol; HMO: hexamethyl disilazane; NaOH: sodium hydroxide; CH₃I, iodomethane; NaBD₄: sodium borodeuteride; NH₄OH: ammonium hydroxide; N: nitrogen; C: carbon; O: oxygen; H₂O: water; CAM: crassulacean acid metabolism; PAAMs: partially methylated starch acetates; WSC: water soluble carbohydrates; DP: degree of polymerization; GC-MS: gas Chromatography-mass spectrometry; MALDI-TOF-MS: matrix assisted laser desorption time-of-flight mass spectrometry.

Identification of a yeast strain as a potential stock whuber: characterization and kinetic characterization

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Keywords: fructose consumption; alcoholic fermentation; *Aspergillus niger*

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Microorganism Degradation Efficiency in BOD Analysis Formulating a Specific Microbial Consortium in a Pulp and Paper Mill Effluent

Luis A. Ordaz-Díaz,^{•3} Juan A. Rojas.-Comreras,¹ Oiga "1 Rutiaga-Quifionl!Si MarrhaR. Morcno,-J mcnc,_ •Felipe Alal!¹istc-.MondrJgón.'and Sergio Yallc-Ccrv,u1tcs'

Pulp and paper mills are a major source of pollution, generating large amounts of intensely colored effluent that goes to the receiving end of a wastewater treatment plant. The biochemical oxygen demand test (BOD₅) relies heavily on the microbial metabolic capability added to the test as seed material. The seeding material in the test is obtained from sewage samples or from commercial sources. Specific organic pollutants that are present in paper and pulp mill effluent can only be degraded by specific microorganisms. Therefore, common sewage or synthetic seed may feed erroneous results. In this study, specific microbial species were selected to evaluate their degradation efficiency both individually and in combination. The microbes, strains selected in the formulated seed exhibit BODs in a reproducible and synergistic manner. The formulation of this specific microbial consortium can be used to develop bioremediation strategies.

Keywords: Paper and pulp; BODs; COD; Degradation; Formulation

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INTRODUCTION

In the pulp and paper industry, combined chemicals have led to water pollution. These chemicals come from the industrial use of fiber, cellulose, and lignin. These components can impart high COD (chemical oxygen demand), color, and BOD₅ to the effluent (Sengh, et al., 2003). The total chemical oxygen demand (COD_T) is approximately 10 times higher than the biological oxygen demand (BOD₅). It is considered a useful quality parameter indicating the degree of biological degradation of organic matter in terms of oxygen consumption (Pappalardo, 1996).

Most of the industrial effluents contain heterotrophic microorganisms that can degrade the organic matter measured by the BOD₅ and chemical oxygen demand (COD_T) tests without adding a microbial seed. The efficiency of these microorganisms to degrade organic matter is measured by the BOD₅ and chemical oxygen demand (COD_T) tests. The time required to reduce the concentration of organic matter obtained directly from the process via solvation and activation improves the treatment (Kumar, 2001).

Trabu et al. (2005) reported that a white rot fungus was isolated from soil samples. This fungus was continuous pulp and paper mill effluent irrigation and identified as *L. chrysostomus*. It is capable of 79% COD reduction. De Oliveira-Jorge, et al. (2009) evaluated the ability of *Bacillus*, *rhizinus*, *Candida* to produce alkanoic acids.

Screening of Native Yeast from *Agave duranguensis* Fermentation for IsoamyJ Acetate Production

Geranio Jlernández-Carbajal¹• Oiga Miri:im Rutiaga-Quiñones¹, Araceli 'rcrc;..."; va²

Gcrardo Saucdo-Castañcd al, Adrianc iVll'd ciros.i. Carlos Ricardo Soccol.... and Nicolás Óscar Soto-Cruz["]

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INTROOUCTI0.'1

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Yeasts Isolated from the Alcoholic Fermentation of *Agave duranguensis* During Mezcal Production

Jesús B. Póez-Lerma¹, Armando Arias-García² Oigo M.
Ruth O. Quíñon¹ Elodio Barrio¹ and Nicolás O. Soto-Guz¹

Durango, Mexico

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Key Words: mezcal; native yeasts; *Saccharomyces cerevisiae*

INTRODUCTION

Fermented beverage have been an important part of Central American culture since ancient times. Mezcal, a traditional Mexican spirit made from the agave plant, has been produced in Durango, Mexico for centuries. The production of mezcal involves the fermentation of agave juice by yeast, which is typically added to the juice after it has been heated to 60°C for 12 hours. This process is known as "cooking" or "cocción".

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Evaluation of *Eichhornia crassipes* as an Alternative Raw Material for Reducing Sugars Production

Héctor A. Filo-Pérez,¹ José G. Rutiaga-Quiñones,¹ Cristobal A.;uilar-González,¹ Jesús D. Pácz,¹ Javier López,¹ and Ol;rn M. Rutia,¹a Quiñones^{1,2}

Water hyacinth was analyzed to determine its the micellulos ellignin content, evaluating the conditions for the saccharification process with commercial microbial enzymes. Plant material, including leaves and stalks, was pretreated at several temperatures (100, 110, and 120 °C) with different sulfuric acid concentrations (0.5, 1.0, 1.5, 2.0, 2.5, and 3%) and residence times (0, 15, 30, 45, 60, 90, and 120 min). Total reducing sugars were measured by the dilute sulfuric acid method. The optimum conditions that maximized the yield of reducing sugars included a pretreatment with 2% (v/v) sulfuric acid at 110 °C for 90 min. The optimum conditions for enzymatic saccharification used the commercial enzyme Cellulase 50 · E for 24 h of hydrolysis. The maximum yield was 0.54 g of fermentable sugars per gram of biomass. Data demonstrated that *E. crassipes* is suitable as a raw material for products such as bioethanol; however, further fermentation studies are required.

Keywords: Bioethanol; Lignocellulose; Pretreatment; Reducing sugar; Water hyacinth

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INTRODUCTION

Water hyacinth, a fast growing aquatic plant, native to Brazil. It is an ornamental plant found in different countries around the world. *Eichornia crassipes* is used in traditional medicine and it removes heavy metals from water bodies (Ganguly *et al.* 2012).

E. crassipes is considered a weed and sometimes a plague around the world; however, it can potentially be a resource due to its high carbohydrate content (18% cellulose, 50% hemicellulose). *E. crassipes* may be an excellent source of sugars, which can be converted to produce ethanol. The bioethanol production is achieved through three steps: pretreatment to liberate cellulose and hemicellulose; hydrolysis of both cellulose and hemicellulose to obtain free sugars, and fermentation conversion of sugars to alcohol (Singh and Bishnoi 2013).

In Mexico, utilization of new lignocellulosic materials for biofuel production is of great importance to the future of bioethanol. The use of lignocellulosic materials for ethanol production is favored by abundance and low cost. *E. crassipes* grows very fast under the climatic conditions present in some regions in Mexico (Kumar and Wyman 2009).

O JGIJ'IAL I'APER

Volatile compound production in *Agave duranguensis* juice fermentations using four native yeasts and NH₄Cl supplementation

O. Iñiriam Kutiaga-Quiñunc · Érica Córdova ·
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Effect of glucose concentration on the rate of fructose consumption in native strains isolated from the fermentation of *Agave duranguensis*

M. Díaz-Campillo · N. Urtfa · Ó. Soto ·
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Introduction

Glucose and fructose are simple sugars found in many foods. The conversion of sucrose by yeast is performed by the enzyme invertase (Arroyo-Lopez et al. 2009). In most studies, the hemicellulose fraction, which contains glucose and galactose, is preferred over the cellulose fraction, which contains glucose and xylose. In general, the proportion of fructose to glucose in the hydrolysate of corn starch is higher than that of sucrose (Babu et al. 2011).

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Resea.ch Al!lc le

Mixing Analysis for a Fermentation Broth of the Fungus *Beauveria bassiana* under Different Hydrodynamic Conditions in a Bioreactor

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Keywords: Bioreactor, Filamentous fungus, Hydromechanics, Numerical simulation, Rheology

DOI: 10.1002/csat.201200130

1 Introduction

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Ful Length Research Paper

***Saccharomyces cerevisiae* strains with robust responses to fermentation stresses isolated from the alcoholic fermentation of *Agave duranguensis* musts**

J. Páez¹, E. Córdova¹, Ó. Soto¹, E. Barriuso², C. Belloch³ and O. M. Rutiaga-Ouiñones¹.

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Vessels used in fermentation processes are subject to different stress conditions, including low pH, high initial substrate concentration, accumulation of toxic compounds (e.g. ethanol), and temperature fluctuations. The fermentation of mescal is conducted under variable environmental conditions throughout the year. One of the most important environmental factors is temperature, as autumn and winter fermentations occur at low temperatures, but fermentation temperatures are much higher in summertime. The aim of this work was to compare the response of *Saccharomyces cerevisiae* strains isolated from agave fermentations to different stress conditions and two different medium culture one mimicking mescal production, using agave must and other synthetic must development for analysis in wine industry. The strains isolated from agave were compared with a commercial strain used in wine elaboration, which exhibits good tolerance to the different stresses found in industrial fermentations. All strains grew in the presence of glucose and fructose irrespective of the sugar concentration, and low pH did not affect the formation of colonies. Differences in growth were observed among the strains at different temperatures and high concentrations of ethanol. Only 28% of the tested strains exhibited good tolerance to high ethanol concentrations, a desirable trait for avoiding stuck fermentations. The strain ITD00185 was able to grow in alcoholic stress condition, consuming sugar and producing ethanol in agave must. This strain shows tolerance to the different stress conditions and may be a useful starter culture for agave fermentation, the potential of the native strains to be used to improve other industrial fermentation processes that involve low temperatures and high ethanol yields.

Key words: Ethanol tolerance, Mescal, native strains.

INTRODUCTION

The physiological conditions of fermentation processes can affect the growth of the microbes that contribute to the breakdown of sugars and production of alcohol. Different geographic regions and their particular environmental conditions can affect the distribution of native microbial

species (Folch et al., 2004). Microorganisms encounter many different stresses during industrial fermentation. Both natural and industrial fermentation are complex processes due to the multitude of conditions that are created during fermentation. Yeast cells, which play a central role in the fermentation process of wine making, are exposed in a physiologically optimal environment during wine production. Throughout this process they are exposed to a variety of different stresses simultaneously (Bauer and Pretorius, 2000).

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Revista Mexicana de Filología

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Palabras clave: optimización, pura de frijol, hidrólisis enzimática, pretratamiento, celulosa.

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* Autor para la correspondencia. E-mail: lloroz@itam.mx.



Study of the Rheological Properties of a Fermentation Broth of the Fungus *Beijeria hassi* in a Bioreactor Under Oil Circulation Conditions

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Ful/ Length Research Paper

Effect of fermentation temperature on chemical composition of mescals made from *Agave duranguensis* juice with different native yeast genera

Martell Nevárez¹, Maria Angelica¹, Córdova Gurrola¹, Erica Estheta¹, López Mirandal, Javier¹, SotoC ruz¹, Nicolás Oscar¹, López Pérez², Mercedes Guadalupe² Rutiaga Quñones¹ and Oiga Miriam¹.

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In this study the fermentation behavior at 18 and 28°C of different native yeast genera on juice from *Agave duranguensis* was evaluated and the volatile compounds produced in the obtained mescals from these fermentations were identified. The fermentative capacity of *Hanseniaspora uvarum*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* increased at 28°C; however the fermentation behavior of *Torulaspora delbrueckii* was not affected by temperature. In the mescals produced by *H. uvarum*, *T. delbrueckii* and *S. cerevisiae* a greater number and abundance of compounds were identified at 18°C than at 28°C. Conversely *K. marxianus* showed a higher production of these compounds at 28 °C. Some compounds Identified in all mescals were z-methyl-1-propanol, 3-methyl-1-butanol, lactic acid, 1-(2-furanyl)-ethanone, furfural, α-terpineol, ethyl phenyl acetate and phenylethyl alcohol. For strains *H. uvarum*, *T. delbrueckii* and *S. cerevisiae*, the most abundant compound identified was 3-methyl 1-butanol at both temperatures. However, for *K. marxianus*, phenylethyl acetate was the most abundant. The non-*Saccharomyces* genera showed exhibt different behaviors to those reported for wine fermentation when the same genera was used. Therefore, they presented a major fermentative capacity and also volatile compounds characteristic of mescals were produced.

Key words: Non-*Saccharomyces*, temperature, volatile compounds, SPME-GCM S

INTRODUCTION

Mescal is a traditional alcoholic beverage from Mexico that is made similarly to tequila. The process begins with

the harvest of Agave after 8 years of growth. At this stage, the plants are cut from their base, obtaining the Agave heart. Next, these are cooked in ovens or autoclaves. During this stage, polysaccharides, mainly fructans, are hydrolyzed by heat into simple molecules such as glucose, fructose and sucrose. The hydrolyzed sugars are extracted from the must by pressing and camed out!he alcoholic fermentabon by native yeasts or selected strains. Finally, the must with ethanol concentration of approximately 3 to 6%(v/v) is distilled to obtain white mescal or young mescal with a concentration of 35

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Abbreviations: HPLC, High performance liquid chromatography; LSD, least significant difference; DVB/CAR/PDMS, divinylbenzene/carboxylic/polydimethylsiloxane; SPME, solid-phase microextraction.