"Año del Centenario de la Promulgación de la Constitución Política de los Estados Unidos Mexicanos"

Oficina: RECURSOS HUMANOS
D.R.H. 140/17.
ASUNTO: Carta de adscripción

MTRO. MANUEL QUINTERO QUINTERO
DIRECTOR GENERAL DEL TECNOLÓGICO
NACIONAL DE MÉXICO
PRESENTE

El que suscribe Jefe del Departamento de Recursos Humanos del Instituto Tecnológico de Durango, por este conducto hace **CONSTAR** que de acuerdo a la documentación existente en los archivos del Dpto de Recursos Humanos, la **C. Dra. Olga Miriam Rutiaga Quiñones**, con RFC **RUQO750216763** y con clave presupuestal E386300.0000036, con status **(10)**, y fecha de ingreso al SNIT el **1 DE FEB DE 2005** cuenta con **11 años** de adscripción a este Instituto.

Se extiende la presente a petición del interesado para los fines legales a que hubiera lugar, en la ciudad de Durango Dgo. a 13 de Marzo de 2017

ATENTAMENTE

"La Técnica al Servicio de la Patria"

ING. JUAN VANEGAS RENTERÍA
JEFE DEL DEPARTAMENTO DE
RECURSOS HUMANOS

CONACYT
Consejo Nacional de Ciencia y Tecnología

El Sistema Nacional de Investigadores otorga a la

DRA. OLGA MIRIAM RUTIAGA QUIÑONES

la distinción de

INVESTIGADOR NACIONAL NIVEL I

Durante el periodo del 1 de enero de 2017 al 31 de diciembre de 2020 en virtud de sus logros en la realización de trabajo de investigación original.

Dra. Julia Tagüeña Parga
Secretaria Ejecutiva del SNI

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Documento firmado electrónicamente.
13 de diciembre de 2016

Ciudad de México, 11 de Julio de 2016
Oficio No. DSA/103.5/16/8799

Rutiaga Quiñones Olga Miriam
Instituto Tecnológico de Durango
Presente

Me complace informarle que el Comité Evaluador externo al PRODEP, de acuerdo con las Convocatorias 2016, resolvió positivamente su solicitud de Reconocimiento a Perfil Deseable.

En consecuencia, la SES acredita que usted tiene el perfil deseable para profesores de tiempo completo.

La acreditación tiene validez por 3 años a partir de esta fecha y servirá para los fines establecidos en la propia convocatoria, en el entendido de que dejar de laborar en esta institución conlleva la cancelación del reconocimiento.

Sin otro particular, aprovecho la oportunidad para enviarle un saludo.

Atentamente

M. en C. María de Jesús Guillermina Urbano Vidales

Directora

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Microbial diversity and biochemical profile of aguamiel collected from *Agave salmiana* and *A. atrovirens* during different seasons of year

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Abstract Aguamiel is a beverage produced by some *Agave* species that is consumed in its fresh or fermented form. Despite its uses and popularity, seasonal effects on its microbial and chemical profiles are unknown. In this study, using aguamiel collected from *A. salmiana* and *A. atrovirens* during different seasons, we identified microorganisms by sequencing the 16S and 18S rDNA genes and determined their chemical profiles. In total, 49 microbial strains were identified (38 bacteria and 11 yeasts). The highest richness and biodiversity were observed during winter and summer. Different lactic acid bacteria and yeast genera with potential industrial applications were identified, such as *Acetobacter*, *Lactobacillus*, *Leuconostoc*, and *Clavispora*. The analysis of the chemical profiles indicated the presence of maltooligosaccharides and fructooligosaccharides, which are associated with human health improvements, during spring in *Agave* aguamiel. Aguamiel can be used in the food industry due to its microbiological and chemical profiles.

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Keywords *Acetobacter* · *Agave* · Aguamiel · Fructooligosaccharide · Lactic acid bacteria

Introduction

Agave plants are distributed throughout the Mexican highlands, specifically in Coahuila, Zacatecas, San Luis Potosí, Colima, Hidalgo, and Puebla states, at altitudes between 1000 and 2460 masl [1]. These plants grow better at locations characterized by semi-dry and dry climates where the temperatures often exceed 40 °C, and they are capable of tolerating extended periods of drought [2]. Some *Agave* species are known as "magueyes pulqueros" because they can be used to produce aguamiel (AM) after removing the central part of the plant. AM is the maguey sap comprising a liquid rich in carbohydrates, minerals, and proteins, which can be consumed fresh or as a fermented alcoholic beverage called "pulque" [3].

Each magueyes pulqueros plant produces between 100 and 250 L of AM, depending on its size, time of "castration" (removal of the inflorescence), species, weather conditions, and production period [4, 5]. Commercially, the first step in AM extraction is removing the central part (meyolote) of the mature plant (8–10 years old), which must be performed before the plant starts flowering to prevent the loss of stored sap. After cutting the meyolote, the wound is left to mature for 2–3 months to ensure that the sugar content of AM is maximized [5].

Different *Agave* species have been used for AM production, including *A. salmiana*, *A. mapisaga*, *A. atrovirens*, *A. americana*, and *A. ferox* [6]. Various bacterium and yeast species with importance in biotechnological processes and the food industry have been detected in AM [7, 8]. These microorganisms confer characteristic flavors

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Regular article

Kinetic, oxygen mass transfer and hydrodynamic studies in a three-phase stirred tank bioreactor for the bioconversion of (+)-valencene on *Yarrowia lipolytica* 2.2ab

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ABSTRACT

The oxidation of (+)-valencene on *Yarrowia lipolytica* 2.2ab in a three-phase partitioning bioreactor using orange essential oil is a promising technology to market natural (+)-nootkatone overcoming both substrate and product inhibitions. The adequate determination and selection of thermodynamic, kinetic, deactivation, hydraulic and mass transport parameters are essential to perform a suitable strategy of scaling-up. This study is aimed at determining these parameters to identify through a regime analysis the mechanisms limiting the bioconversion process. The volumetric oxygen transfer coefficient (k_La) and the *Sauter* mean drop diameter (d_{32}) values ranged from 10 to 116 h^{-1} and 8 to 18 μm, respectively. The substrate (k_S) and product (k_P) global interfacial mass transfer coefficients were determined from a modified Lewis cell. The k_S and k_P values ranged from 0.6 to 3.0×10^{-5} and 2 to 3×10^{-5} m s^{-1}, respectively. Finally, a kinetic model, considering a bi-substrate reaction and accounting for cell deactivation, was developed. The affinity constants for oxygen and (+)-valencene were $K_{O2} = 7.11 \times 10^{-2}$ mg_{O2} L^{-1} and $K_S = 7.865 \times 10^{-3}$ mg_S L^{-1}, respectively, while catalytic constant related to (+)-nootkatone formation was $k_{cat} = 5.025 \times 10^3$ mgP mg_E^{-1} h^{-1}. Thus, the regime analysis in terms of the characteristic times suggested that kinetics, namely the consumption rate of (+)-valencene was the main mechanism limiting the bioconversion process.

1. Introduction

The compound (+)-nootkatone is a valuable sesquiterpenoid, which possesses an intense grapefruit-like taste that is exploited by the fragrance and flavour industries [1]. Industry and academia are interested in the production of (+)-nootkatone *via* a biocatalytic process using whole cells since this organic compound can be marketed as a natural product. However, the adequate design of this technology requires the selection of optimal cellular enzymes and engineering to carry out the proper scaling-up. Several efforts have focused on enhancing the production of (+)-nootkatone from (+)-valencene using either classical genetics [2] or metabolic engineering [3,4]. However, bioconversions have been mainly affected by substrate and product inhibitions since most of these studies have been performed in two-liquid-phase systems. Notwithstanding, De Gonzalo et al. [5] have proposed the use of organic solvents as a second liquid phase in order to overcome substrate and product inhibitions.

The use of essential oils has been reported as a safe alternative for the bioconversion of pharmaceuticals and terpenes [6]. We have previously demonstrated the successful bioconversion of (+)-valencene to (+)-nootkatone with *Yarrowia lipolytica* 2.2ab in a three-phase partitioning bioreactor using orange essential oil as the dispersed phase [7]. Orange essential oil was used as both substrate reservoir and *in situ* extraction agent in the partitioning

Physicochemical Characterization of Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms)

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Water hyacinth (*Eichhornia crassipes*) is an aquatic flowering plant that belongs to the Pontederiaceae family. The plant is a freshwater hydrophyte that grows in subtropical and tropical regions of the world. The objective of this study was to determine the physicochemical characterization of roots, stems, and leaves of *E. crassipes*. The pH, ash, 1% alkali solubility, extractives, lignin, holocellulose, tannins, and calorific value were determined. Our results showed that the mineral content is relatively high, whereas that for lignin and tannins is low. The pH is moderately acid, and the soluble substances easily dissolved in alkali or organic solvents. Potassium, calcium, and silicon are the major constituents present in the ash of this plant. The determined calorific value was approximately 14.4 MJ/kg.

Keywords: pH; Ash; Extractives; Lignocellulosic material; Calorific value

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INTRODUCTION

The water hyacinth (*Eichhornia crassipes* (Mart.) Solms) is an aquatic flowering plant that belongs to the Pontederiaceae family. The plant is a freshwater hydrophyte that grows in subtropical and tropical regions of the world. Sometimes the water hyacinth is considered an undesirable weed; but various studies have reported its uses, such as in the production of ethanol (Manivannan *et al*. 2012; Awasthi *et al*. 2013; Fileto-Pérez *et al*. 2013; Manivannan and Narendhirakannan 2014); as an adsorbent for heavy metals present in polluted water (Murithi *et al*. 2014); for phytoremediation (Vijetha *et al*. 2014); as a raw material for the production of biogas (Ochieng and Kaseje 2014); as a biofuel (Bergier *et al*. 2012); and as a protein supplement in ruminant feed (Mako *et al*. 2011). Other works have focused on the effect of extractives on its antimicrobial activity (Tharamaiselvi and Jayanathi 2012), or its potential to provide phytosterols for the pharmaceutical industry (Fileto-Pérez *et al*. 2015). The objective of this study was to determine the physicochemical characterization of roots, stems, and leaves of *E. crassipes* with the aim of providing a basis for future applications.

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

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ABSTRACT

Agave juice is fermented and distilled twice in order to obtain mezcal, a traditional spirit beverage. The goal of this study was to select yeast or yeast mixtures that could be used in industrial production of high-quality mezcal. Yeasts isolated during fermentation of *Agave duranguensis* were tested to identify the best strains with respect to the chemical and organoleptic characteristics of the produced mezcal. Three individual yeast strains were selected for further study: *Torulaspora delbrueckii* strain ITD-00014a, *Saccharomyces cerevisiae* strain ITD-00185, and *Kluyveromyces marxianus* strain ITD-00147. Analysis of the response surfaces permitted identification of three inoculants: one mixture (75% *S. cerevisiae* ITD-00185 and 25% *T. delbrueckii* ITD-00014a) and two pure strains (100% *K. marxianus* ITD-00147 and 100% *S. cerevisiae* ITD-00185). Mezcal made by using the inoculant constituted by 75% *S. cerevisiae* ITD-00185 and 25% *T. delbrueckii* ITD-00014a (named mezcal 75*Sc*/25*Td*) was the best in terms of yield, richness of volatile compounds, and acceptability in sensory tests. We propose using this mixture of yeasts to develop a product that can be used as an inoculant in industrial production of mezcal and others spirits from different agave species.

1. Introduction

Fermentation proceeds spontaneously during production of mezcal by artisan methods (Soto-García et al., 2009). It is during this stage that soluble sugars are transformed into ethanol and other compounds. The first days of alcoholic fermentation are dominated by non-*Saccharomyces* yeasts from the genera *Candida*, *Kluyveromyces*, *Torulaspora*, and *Pichia*, among others (Páez-Lerma et al., 2013). These yeasts have low fermentative capacity but generate various flavor compounds, including terpenoids, esters, higher alcohols, glycerol, acetaldehyde, acetic acid, and succinic acid (Jolly et al., 2015; Moreira et al., 2005). After these first days of fermentation, non-*Saccharomyces* yeasts are replaced by strains of *Saccharomyces cerevisiae*, which complete fermentation (Páez-Lerma et al., 2013). Spontaneous fermentation during artisan production of mezcal has two undesirable characteristics: the fermentation time can vary from 3 to 9 days, and the concentration of residual sugar is variable. A starter culture (inoculant) can be used to standardize the fermentation process and to achieve better utilization of the fermentable sugars.

In recent years, some studies have used monocultures or co-cultures of *S. cerevisiae* and non-*Saccharomyces* yeast to produce wine (Andorrà et al., 2012; Benito et al., 2011; Ciani et al., 2010; Sun et al., 2014), mango wine (Sadineni et al., 2012), and a sugarcane and pineapple beverage (Silva et al., 2015). Non-*Saccharomyces* yeasts contribute to the increased production of polysaccharides and can modulate the final concentrations of acetic acid and volatile compounds (Domizio et al., 2011). Moreover, it has been suggested that *Saccharomyces* and non-*Saccharomyces* yeasts may act synergistically (Ciani et al., 2010). For example, mixed cultures of selected yeasts have been developed as starter inoculants to enhance the quality of wine (Benito et al., 2011; Ciani et al., 2010; Esteve-Zarzoso et al., 2000; Pretorius and van der Westhuizen, 1991). Selection of the appropriate strains is generally based on criteria such as fermentative power, tolerance to ethanol, ethanol yield, killer phenotype, and low acetic acid production (Suárez-Lepe and Morata, 2012).

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Whole cell bioconversion of (+)-valencene to (+)-nootkatone by *Yarrowia lipolytica* using a three phase partitioning bioreactor

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Abstract

BACKGROUND: Low permeability of substrates across the cell membrane, cofactor regeneration and product inhibition are some drawbacks of (+)-nootkatone bioconversion. The aim of this work was to evaluate and enhance the bioconversion of (+)-valencene to (+)-nootkatone with *Yarrowia lipolytica* in a partitioning bioreactor using orange essential oil as the dispersed phase.

RESULTS: Preliminary experiments in shake flasks allowed enhancing (+)-nootkatone bioconversion to obtain favorable operating conditions (0.2% w/v of CTAB, 2.0 mmol L^{-1} of niacin and 11.5 g L^{-1} of biomass) to produce 420.9 mg L^{-1}. Bioreactor experiments in a two-phase system using 0.2% (w/v) of CTAB, 2.0 mmol L^{-1} of niacin and 22.5 g L^{-1} of biomass produced a maximum (+)-nootkatone concentration of 619.8 mg L^{-1} which was around the product inhibition concentration. Nevertheless, the partitioning three-phase system using orange essential oil overcame product inhibition, obtaining concentrations up to 852.3 mg L^{-1}.

CONCLUSIONS: This is the first report of a wild type *Y. lipolytica* with the enzymatic machinery to carry out this bioconversion. The multiphase partitioning bioreactor concept seems to have good potential for enhancing the productivity of (+)-nootkatone. The bioconversion approach presents an attractive way to produce and recover (+)-nootkatone *in situ* using a natural (+)-valencene source.

Keywords: bioconversion; partitioning bioreactor; (+)-nootkatone; orange essential oil; yeast; product inhibition

INTRODUCTION

The compound (+)-nootkatone is a sesquiterpenoid which possesses an intense grapefruit-like taste and other valuable properties that are exploited by the fragrance and flavour industries.[1] The production of (+)-nootkatone is performed via chemical synthesis, mainly from the sesquiterpene (+)-valencene, which is readily available from the orange industry and also through the use of environmentally unfriendly oxidising agents, such as tert-butyl peracetate[2] and tert-butyl hydroperoxide in combination with catalytic metal supported on silica.[3] However, the resulting (+)-nootkatone produced via chemical synthesis cannot be marketed as a 'natural' product and does not satisfy increasing market demands for natural aromatic compounds. In order to meet this demand, efforts have been focused on the use of biotechnological processes with bacteria, fungi or plants.[4] Some researchers have investigated genetically modified microorganisms, and several published reviews[5,6] have provided an overview of recently acquired knowledge about whole cell bioconversions and future industrial applications. In particular, industry and academia are interested in a biocatalytic process using whole cells which involves the selection of an optimal cellular enzyme, reaction engineering, product recovery and scaling-up.

Gavira *et al.*[7] reported a bioconversion process for (+)-nootkatone production using plant enzymes expressed in *Saccharomyces cerevisiae*. However, the process was inhibited by both the substrate (>4 g L^{-1}) and the product (1.0 g L^{-1}) as well

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GC/MS Analysis of Some Extractives from *Eichhornia crassipes*

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Eichhornia crassipes (water hyacinth) is an invasive weed that causes serious issues for rivers, lakes, and other reservoirs around the world, although it can be an excellent source for bioactive compounds such as phytosterols and some steroids found in many plants. In this study, water hyacinth samples from both Durango and Distrito Federal in Mexico were collected. Ascendant extracts (cyclohexane, hexane, acetone, and methanol) from their leaves, stems, and roots were analyzed. Using boron trifluoride (~10% [~1.3 M] in 1-butanol), all extracts were derivatized. Twenty-four derivatized samples were analyzed using a gas chromatography-mass spectrometry (GC/MS) method. Twenty carboxylic acids were found, as well as squalene, which was found in nine extract samples: four cyclohexane extracts, one hexane extract, three acetone extracts, and two methanol extracts. A compound not reported before, β-stigmasterol, was identified on three hexane extracts, an acetone extract, and a methalonic extract. Spirostane in acetone root extract and cholestane in cyclohexane stem-leaf extract were also identified.

Keywords: Extractive substances; Steroids; GC/MS; Water hyacinth

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INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is a plant from Brazil that has been spread around the world by man. It is considered an invasive weed; however, it could be an excellent source of chemical compounds because of its chemical composition, which includes chemical species that can be utilized for several applications. At present, this plant has been detected in more than 62 countries, and it is considered the most important floating weed in tropical and subtropical regions (Groote *et al.* 2003). The main issues caused by *E. crassipes* is that it blocks navigation pathways; decreases oxygen in water, causing fish species to die; and causes eutrophication in rivers and lakes (Dandelot *et al.* 2008). This aquatic plant has the highest growing rate in the world, providing 0.26 tons of dried biomass per hectare in all seasons. Because of global warming, which has caused a rise in global temperatures, this plant has spread to higher latitudes (Huber *et al.* 2006; Hellmann *et al.* 2008).

Accepted Manuscript

Screening of microorganisms for bioconversion of (+)-valencene to (+)-nootkatone

D.M. Palmerín-Carreño, O.M. Rutiaga-Quiñones, J.R. Verde Calvo, A. Prado-Barragán, S. Huerta-Ochoa

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BIOCONVERSION OF (+)-NOOTKATONE BY *Botryodiplodia theobromae* USING A MEMBRANE AERATED BIOFILM REACTOR

BIOCONVERSIÓN DE (+)-NOOTKATON POR *Botryodiplodia theobromae* UTILIZANDO UN REACTOR DE BIOPELÍCULA DE MEMBRANA AIREADA

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Abstract

The aim of this work was to evaluate the bioconversion of (+)-valencene to (+)-nootkatone by *B. theobromae* using a membrane aerated biofilm reactor (MABR) in a two liquid phase system with orange essential oil as the organic phase. In the aqueous phase system, a (+)-nootkatone production rate up to 3.98 mg L^{-1} h^{-1} was achieved, obtaining a final product concentration of 398.08 mg L^{-1} with a bioconversion of 62 %. A two liquid phase system, using orange essential oil as the dispersed phase, was also studied and a final (+)-nootkatone concentration of 310.37 mg L^{-1} was achieved in the organic phase, with a bioconversion of 30.5 % and a production rate of 2.46 mg L^{-1} day^{-1}. The lower performance obtained using the two phase system was probably due to mass transfer limitations. The present work is the first report on an MABR for the bioconversion of (+)-valencene to (+)-nootkatone. Further studies on bioconversion products and optimization of biofilm reactor operations are needed to enhance bioconversion.

Keywords: bioconversion, (+)-nootkatone, *Botryodiplodia theobromae*, orange essential oil, membrane aerated biofilm reactor.

Resumen

El objetivo de este trabajo fue evaluar la bioconversión de (+)-valenceno a (+)-nootkaton por *B. theobromae* usando un reactor de biopelícula de membrana aireada (MABR) en un sistema de dos fases líquidas con aceite esencial de naranja como fase orgánica. En el sistema de fase acuosa, se logró una tasa de producción de (+)-nootkaton de hasta 3.98 mg L^{-1} h^{-1}, obteniendo una concentración de producto final de 398.08 mg L^{-1} con una bioconversión de 62 %. También se estudió un sistema de dos fases líquidas, utilizando aceite esencial de naranja como fase dispersa, y se alcanzó una concentración final de (+)-nootkaton en la fase orgánica de 310.37 mg L^{-1}, con una bioconversión de 30.5 % y una tasa de producción de 2.46 mg L^{-1} $día^{-1}$. El menor rendimiento obtenido mediante el sistema de dos fases fue probablemente debido a las limitaciones de transferencia de masa. El presente trabajo es el primer reporte utilizando un MABR para la bioconversión de (+)-valenceno a (+)-nootkaton. Se necesitan estudios adicionales sobre los productos de bioconversión y la optimización de las condiciones de operación del reactor de biopelícula para mejorar la bioconversión.

Palabras clave: bioconversión, (+)-nootkaton, *Botryodiplodia theobromae*, aceite esencial de naranja, reactor de biopelícula de membrana aireada.

1 Introduction

The compound (+)-nootkatone is a sesquiterpenoid which possesses an intense grapefruit-like taste and other valuable properties that are highly appreciated by the fragrance and flavor industries (Ladaniya, 2010). The production of (+)-nootkatone is performed via chemical synthesis, mainly from the sesquiterpene

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