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LIPID PRODUCTION BY *Penicillium decumbens* FROM THE DIRECT CONVERSION OF SEAWEED BAGASSE

PRODUCCIÓN DE LÍPIDOS POR *Penicillium decumbens* A PARTIR DE LA CONVERSIÓN DIRECTA DE BAGAZO DE MACROALGAS

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Abstract
A wide variety of microorganisms produce lipids of which filamentous ascomycetes fungi have the advantage of directly using cellulosic by-products as primary carbon source to produce them. The capacity of *Penicillium* to accumulate mycelial lipids has been recognized for decades. But, their potential as lipid producer from cellulosic substrates is limited. This study deals with the lipid production from a cellulosic substrate (seaweed bagasse) by the new isolate *Penicillium decumbens* M11. This fungus produces 6 mg of total lipids per gram of seaweed bagasse (mg/g). When a 2^3 factorial design was applied to study the effects of temperature, water fraction, and C/N ratio on lipid production, the fungus increased their lipid accumulation to 97 mg/g at 28°C, a C/N ratio of 50 and a water fraction of 50%. Palmitic, oleic, linoleic, and stearic acids were the most abundant fatty acids.
Keywords: ascomycetes, macroalgae waste, microbial lipids, solid state fermentation.

Resumen
Existe una gran diversidad de microorganismos que producen lípidos. Entre ellos, los hongos filamentosos tienen la ventaja de utilizar subproductos celulósicos como fuente de carbono para producirlos. Por décadas se ha reconocido la capacidad de *Penicillium* como acumulador de lípidos, pero poco se conoce sobre su capacidad de producir lípidos a partir de sustratos celulósicos. Este estudio presenta la producción de lípidos a partir de un sustrato celulósico (bagazo de macroalgas) por un nuevo aislado fúngico *Penicillium decumbens* M11. Este hongo produce 6 mg de lípidos totales por gramo de bagazo de macroalgas (mg/g). Después de aplicar un diseño factorial 2^3 para estudiar los efectos de temperatura, contenido de agua y relación C/N sobre la producción de lípidos, el hongo incrementó su potencial de acumulación a 97 mg/g a 28°C, una relación C/N de 50 y 50% de contenido de agua. Los ácidos grasos más abundantes fueron el ácido palmítico, oleico, linoleico, y esteárico.
Palabras clave: ascomicetos, macroalgas, lípidos microbianos, fermentación en estado sólido.

1 Introduction

In the last years, a growing number of studies have focused their efforts on producing microbial lipids from different carbon sources that, depending on the lipid profile, are intended for biodiesel fuel, nutraceuticals or therapeutic compounds. This area of research is divided into two major microbial groups, autotrophic microalgae and heterotrophic microorganisms such as bacteria, yeasts and filamentous fungi (Robles-Hereida *et al*., 2016; Thevenieau and Nicaud, 2013). Lipid production bioprocesses that are mediated by heterotrophic microorganisms have the advantages of using fermenters with mature technologies that do not depend on light, as microalgal bioreactors do, and using a wide variety of carbon sources. The selection of such carbon sources is of concern for industrial applications because the feedstock cost has a great impact on the total production cost (Ferreira *et al*., 2016). Accordingly, some authors have explored various plentiful, low-value by-products to perform microbial lipid production. Some examples include lignocellulosic hydrolysates, crude glycerol, end products of fermentation, mill effluents, animal fats, and waste oils (Donot *et al*., 2014). These liquid carbon sources are very convenient

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for submerged fermenters, but, there is also great potential in solid organic wastes that have enough polysaccharides to be converted into microbial lipids. This usually means that the hydrolysis of insoluble polysaccharides using energy-demanding processes or detoxification of hydrolysates is mandatory (Kamat *et al*., 2013; Thevenieau and Nicaud, 2013). Further, some oleaginous microorganisms with hydrolytic capacities could simultaneously perform carbohydrate solubilization and lipid synthesis (Singh, 1991; Hiu *et al*., 2010; Kamat *et al*., 2013; Wei *et al*., 2013; Cheirsilp and Kitcha, 2015; Kitcha and Cheirsilp, 2014).

For decades, Ascomycetes have been known as enzyme producers, depending on the substrate on which they grow, and some filamentous ascomycetes fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* have been recognized as oleaginous fungi (Koman *et al*., 1969; Bhatia *et al*., 1972; Abraham and Srinivasan, 1984; Papanikolaou *et al*., 2010; Donot *et al*., 2014; Ferreira *et al*., 2016). For example, to complete the dual task of polysaccharide hydrolysis and lipid production, Singh (1991) grew the cellulolytic *Aspergillus niger* AS-101 on cellulose for lipid accumulation. Hui *et al*. (2010) used the cellulolytic *Aspergillus oryzae* A-4 to produce lipids from wheat straw. Kamat *et al*. (2013) produced lipids from acid-treated sugarcane bagasse using *Aspergillus terreus*, whereas Kitcha and Cheirsilp (2014) used *Aspergillus tubingensis* to produce lipids from palm pressed fiber and empty palm fruit bunches. Also, Cheirsilp and Kitcha (2015) used lignocellulosic wastes from a palm oil mill as the carbon source for lipid synthesis mediated by *Aspergillus tubingensis* TSIP9. The range of lipid accumulation reported in these studies was 37 to 89 mg of total lipids per g of solid feedstock. Presently, *Aspergillus* is the most studied filamentous fungi for lipid production, despite the great diversity of Ascomycetes. In particular, *Penicillium* is one of the most abundant and widely distributed microfungi in nature and, in conjunction with *Aspergillus*, has made a great contribution to the white biotechnology with multiple applications at the industrial scale (Donot *et al*., 2014). For many decades, *Penicillium* has been identified as an oleaginous fungus (Koman *et al*., 1969; Bhatia *et al*., 1972; Abraham and Srinivasan, 1984; Hamid *et al*., 1987; Lomascolo *et al*., 1994; Suutari, 1995). But, to date, there are no studies on the potential for lipid production by *Penicillium* growing on solid substrates.

On the other hand, seaweeds are macroscopic multicellular marine macroalgae that naturally grow attached to hard substrata that are part of coastal ecosystems. They are distinguished by very high growth rates and biomass productivity (Borines *et al*., 2011). Each macroalgae has a distinct carbohydrate profile: green algae contain starch, pectin, and cellulose; brown algae harbor large portions of laminarin and mannitol as well as alginate, cellulose, and fucoidan; and red algae have agar, carrageenan, funoran, and small portions of cellulose. The dry carbohydrate fraction in seaweeds is 42% to 73%, depending on the seasonal, environmental conditions and the macroalgae species (Hong *et al*., 2014). Seaweeds such as *Gelidium*, *Gracilaria*, *Chondrus*, *Euchema*, and *Gigartina* are used as food, animal feed, fertilizer supplement, and primarily as raw material for the extraction of phycocolloids (agar, carrageenan, furcellaran, and algin). According to recent data, the worldwide seaweed production was 26 million tons of wet weight (FAO, 2014). From this biomass, after industrialization, at least 25% of the dry mass constitutes solid wastes (Kumar *et al*., 2013). Thus, this portion of macroalgae containing residual polysaccharides could be intended for microbial lipid production in temperate coastal regions. The aim of this study was to explore the potential of a novel strain of *Penicillium* isolated from an intertidal zone for direct lipid production using seaweed bagasse.

2 Materials and methods

2.1 Microorganism and screening

The *Penicillium decumbens* M11 (Fig. 1A) in conjunction with another eight strains were isolated using conventional microbiological procedures from Agua Caliente beach (an active tectonic element with hot springs) in Baja California, Mexico (31°43'14"N, 116°39'29"W). Only the fungus *P. decumbens* M11 was molecularly identified by amplifying the internal transcribed spacer according to previously reported methods (Saitou and Nei, 1987; White *et al*., 1990; Tamura *et al*., 2007). The resulting sequence was deposited into the GenBank database under accession number JN164554. The fungus was maintained on potato dextrose agar at 4°C. *P. decumbens* M11 was first tested for lipid accumulation on potato dextrose broth (PDB) and a nitrogen-limited medium, N-LM (composition in grams per liter: 30 glucose; 0.08 $(NH_4)_2SO_4$; 7 KH_2PO_4; 2 Na_2HPO_4; 1.5 $MgSO_4 \cdot 7H_2O$; 0.1 $CaCl_2 \cdot 2H_2O$; 0.008 $FeCl_3 \cdot 6H_2O$;

0.001 $ZnSO_4 \cdot 7H_2O$; 0.0001 $CuSO_4 \cdot 5H_2O$; 0.0001 $Co(NO_3)_2 \cdot H_2O$; 0.0001 $MnSO_4 \cdot 5H_2O$). The cultures were incubated at 28°C for 3 days with orbital agitation at 80 rpm. Fungal biomass was recovered by centrifugation (6000 × g/10 min) from 50-mL broth, and then dried (60°C/24 h) using a convection oven. The lipid droplets accumulated in the mycelium were microscopically examined with Black Sudan B stain (Sigma-Aldrich 199664) according to the method reported by Burdon (1946), and the total lipids were extracted from the fungal biomass according to Bligh and Dyer (1959).

2.2 *Lipid synthesis from seaweed solid wastes*

2.2.1 *Substrate*

A 4-kg mixture of seaweed bagasse was obtained from a local producer of hydrocolloids that industrializes *Gelidium* and *Gracilaria* for agar production and *Chondrus crispus*, *Euchema cottonii*, *Euchema spinosum*, and *Gigartina* for carrageenan production (Agarmex). The seaweed bagasse was dried at 60°C for 24 h and milled using a laboratory hammer mill to obtain 20-mm particles. The milled material was stored indoors in an opaque plastic container for already one month in an air-conditioned laboratory (22°C and 42% relative humidity).

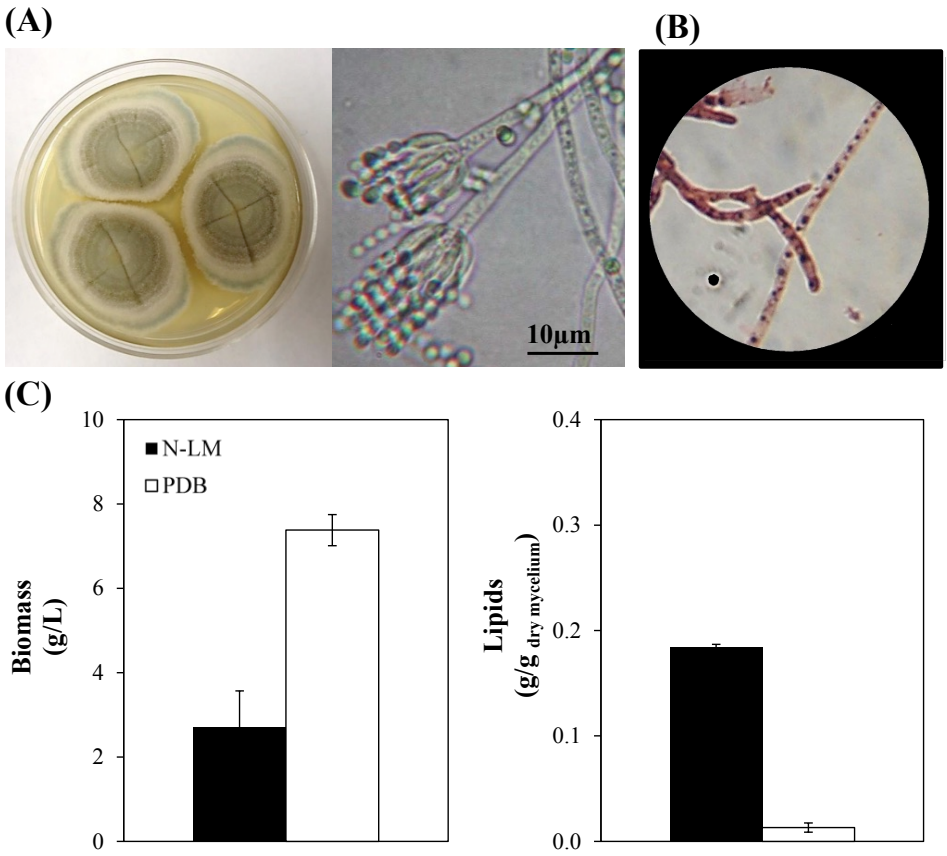

Fig. 1. Panel A. Colonial appearance (7 days) and light microscopic appearance of *Penicillium decumbens* M11. Panel B. Fungal mycelia of *Penicillium decumbens* M11 growing on a nitrogen-limited medium (magnification at 100×). Liposoluble granules were stained with Black Sudan B and contrasted with safranine. Panel C. Biomass and mycelial lipids extracted from fungal cultures grown on potato dextrose broth (PDB) and a nitrogen-limited medium (N-LM). Bars indicate standard errors (n = 3).

The components of the seaweed bagasse on a per kilogram basis were determined using previously reported methods (Sattler and Zerban, 1948; APHA, 1992): 73.9 ± 6.6 volatile solids; 34.3 ± 2.0 organic carbon; 64.3 ± 3.5 total carbohydrates; 20.2 ± 3.0 protein; 26.1 ± 1.8 ash; 0.3 ± 0.06 total Kjeldahl nitrogen; and 0.47 ± 0.04 lipids. According to previous studies, it is expected that the major portion of the carbohydrates present in the seaweed bagasse is composed by cellulosic sugars (Kumar *et al*., 2013; Hong *et al*., 2014).

2.2.2 *Lipid accumulation pattern*

P. decumbens M11 was grown on seaweed bagasse to determine their lipid accumulation pattern and set the time at which the highest lipid accumulation was attained. The fungal biomass from a preculture (7×10^8 spores in 75 mL PDB for 7 days at 28°C) was recovered by decantation, washed with sterile water, and added to 250-mL Erlenmeyer flasks that contained 5 ± 0.1 g of sterile seaweed bagasse with an 80% (w/w) water content at pH 6.0. The flasks were incubated statically at 28°C. Every 24 h, three Erlenmeyer flasks were examined to determine the lipid accumulation over 10 days. One treatment with seaweed bagasse alone was used as a control, and the lipids that it contained were subtracted from those in the fungal cultures.

2.3 *Experimental design for lipid accumulation*

A 2^3 factorial design was used to study the effects of C/N ratio (50 and 80), water mass fraction (50% and 80%), and temperature (21°C and 28°C) on the total lipid synthesis and the fatty acid profile in *P. decumbens* M11. To this end, sterile dry seaweed bagasse (5 ± 0.1 g) were mixed with the fungal biomass that was obtained by decantation from a preculture (7×10^8 spores in 75 mL PDB for 7 days at 28°C) into 250-mL Erlenmeyer flasks. Then, the water content (% w/w) and C/N ratio were adjusted with a modified N-LM at pH 6.0 according to the factorial design. The flasks were incubated statically for 4 days. One treatment with seaweed bagasse alone was used as a control, and the lipids that it contained were subtracted from those in the fungal cultures. All experiments were performed in triplicate, and the results were presented as the mean ± SD (n = 3).

2.4 *Analytical methods*

After incubation, the total lipids were extracted according to Bligh and Dyer (1959) and expressed as extracted lipids per gram of dry seaweed bagasse (mg/g). Then, the extracted lipids were transesterified with methanol and NaOH. The resulting fatty acid methyl esters were analyzed using a gas chromatograph (Varian CP-3380) equipped with an Omegawax^{TM} 250 silica column (30 m x 0.25 mm x 0.25 μm). Nitrogen was used as the carrier gas. The column temperature was programmed from 120°C to 190°C, increasing 10°C/min. The injection port and detector were maintained at 250°C and 270°C, respectively.

2.5 *Statistical analysis*

The data were examined by analysis of variance (ANOVA, $p < 0.05$; Montgomery, 1991) to determine the effect of each factor and its interactions on total lipid production and the fatty acid profile using the software Design-Expert 10 (Stat-Ease, Inc, Minneapolis, USA).

3 Results and discussion

3.1 *Screening for lipid accumulation*

P. decumbens M11, in conjunction with another eight fungal species, were isolated from estuarine sediments in Baja California, Mexico. These nine fungi were tested for lipid accumulation using PDB and an N-LM. From them, only five of the fungi accumulated lipids in mycelia, and *P. decumbens* M11 outperformed all the fungal species (Fig. 1B). It grew almost three times more on PDB than N-LM but the lipid synthesis was almost ten times more on N-LM than PDB (Fig. 1C). After 3 days of incubation, the mycelial lipid accumulation in *P. decumbens* M11 was 19%. This mycelial lipid accumulation behavior was similar to those reported for *Penicillium camemberti*, *Penicillium frequentans*, *Penicillium lilacinum*, and *Penicillium roqueforti* which ranged from 5% to 22%, and to the behavior of other filamentous ascomycetes fungi such as *A. oryzae* A-4, which ranged from 15% to 18% (Abraham and Srinivasan, 1984; Hamid *et al*., 1987; Lomascolo *et al*., 1994; Hiu *et al*., 2010).

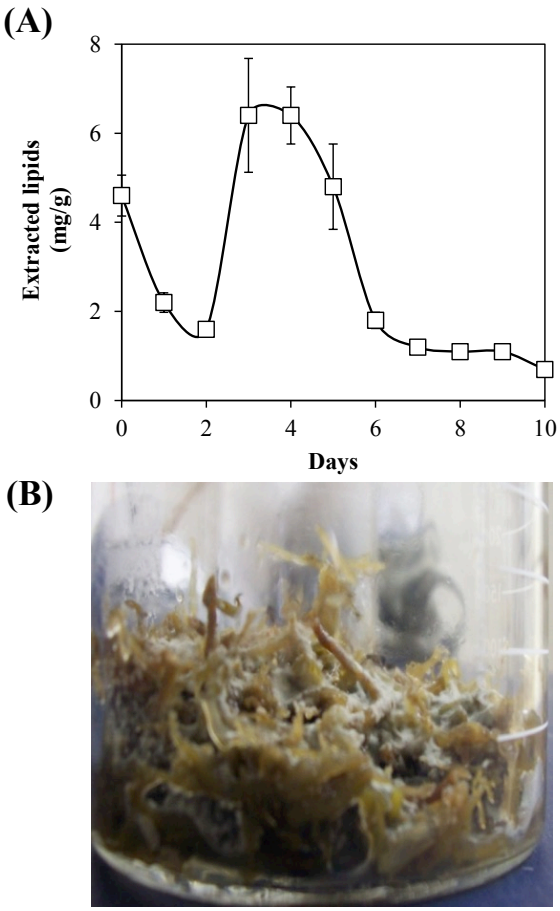

Fig. 2. Panel A. Lipid accumulation pattern of *Penicillium decumbens* M11 grown on seaweed bagasse. Bars indicate standard errors (n = 3). Panel B. Appearance of *Penicillium decumbens* M11 grown on seaweed bagasse.

3.2 *Lipid accumulation pattern from seaweed solid wastes*

P. decumbens M11 was grown on seaweed bagasse without nutrient addition and the resulting lipid accumulation pattern is shown in Fig. 2A. Three stages were observed: i) lipids already present in the fungal biomass decreased rapidly during the first two days of incubation; ii) then, mycelial lipids peaked at 6.4 ± 0.2 mg/g on day 3 and remained for two days; and iii) finally, accumulated lipids were consumed at the end of incubation. A similar lipid accumulation pattern was observed with *A. oryzae* A-4 grown on a mixture of wheat straw and bran (Hiu *et al.*, 2010). These authors argued that lipids were consumed during the first stage because the available sugars in the substrate were insufficient; these sugars are used to synthetize hydrolytic enzymes to degrade the solid substrate. Then, when the enzymes have been excreted, the sugars are released and the fungus grows, accumulating lipids. Finally, when the solid substrate has been consumed, the accumulated lipids are catabolized. Based on the lipid accumulation pattern shown in Fig. 2A, the time at which *P. decumbens* M11 accumulates the highest content of mycelial lipids was determined to be four days.

3.3 *Effects of water content, C/N ratio and temperature on lipid accumulation and profile*

Table 1 shows the average values of lipid accumulation for each treatment. ANOVA showed that the effects of C/N ratio, water content, and temperature on lipid accumulation in *P. decumbens* M11 were statistically significant ($p < 0.0001$; Table 2). The interaction of factors, water content and temperature also had a significant effect on the dependent variable ($p < 0.05$). The empirical relation in terms of actual factors is presented in Eq. 1 (only significant terms are showed):

$$\begin{aligned}\text{Total lipids (mg/g)} = {} & -151.2 - 2.2 * \text{C/N ratio} + \\ & 1.5 * \text{water content} + 14.5 * \text{Temp} \\ & -0.1 * \text{water content} * \text{Temp} \quad (1)\end{aligned}$$

The value of R^2 was 0.82, consistent with the adjusted R^2 of 0.79 ($R^2 > 0.75$ indicates the aptness of the model), ensuring adequate adjustment of the experimental data to the model.

Of the tested conditions, the water content had a very significant effect on lipid production from seaweed bagasse by *P. decumbens* M11 ($p < 0.0001$). Figure 3A shows the main effect of water content on lipid accumulation, where on average the total lipids per gram of dry substrate were up to 60 mg/g at 50% water, whereas approximately 30 mg/g were obtained at 80% water. Free water is a critical parameter in solid-state fermentation (SSF) and affects heat and mass transfer (metabolic heat, oxygen, sugars, and toxic metabolites), swelling, and substrate porosity. Growth, substrate consumption, and metabolite production are affected by free water. In this study, a water mass fraction of 50% was sufficient to swell the seaweed bagasse and, allow the fungus to colonize, hydrolyze, and consume nutrients. Conversely, a water mass fraction of 80% appears to reduce substrate porosity and cause aggregation of solid particles, creating anaerobic zones in which

Table 1. 2^3 factorial design to study the effects of C/N ratio, water fraction and temperature on total lipid production from seaweed bagasse by *Penicillium decumbens* M11.

Factors			Total lipids
C/N	**Water fraction (%)**	**Temperature (°C)**	**(mg/g)**
50	50	21	55.3 ± 3.9
80	50	21	54.0 ± 7.8
50	80	21	42.9 ± 8.1
80	80	21	28.5 ± 2.5
50	50	28	96.7 ± 1.1
80	50	28	73.3 ± 13.9
50	80	28	42.0 ± 9.6
80	80	28	28.5 ± 3.2

Table 2. Statistical analysis of factorial design with three variables for lipid accumulation in *Penicillium decumbens* strain M11 grown on seaweed solid waste.

Significant variables	Sum mean values	Degree of freedom (df)	Mean square	F-value	Probability (P)
Model	0.056	7	3.77 E-003	8.16	<0.0001
A. C/N ratio	4.63E-03	1	4.63 E-003	10.01	0.006
B. Water content	0.041	1	0.041	89.16	<0.0001
C. Temperature	2.86 E-003	1	2.86 E-003	6.2	0.024
Interaction BC	3.76 E-003	1	3.76 E-003	8.13	0.012
Error	7.40 E-003	8	4.63 E-003	0.011	
Total	0.064	15			

Notes: only the significant interaction is shown.

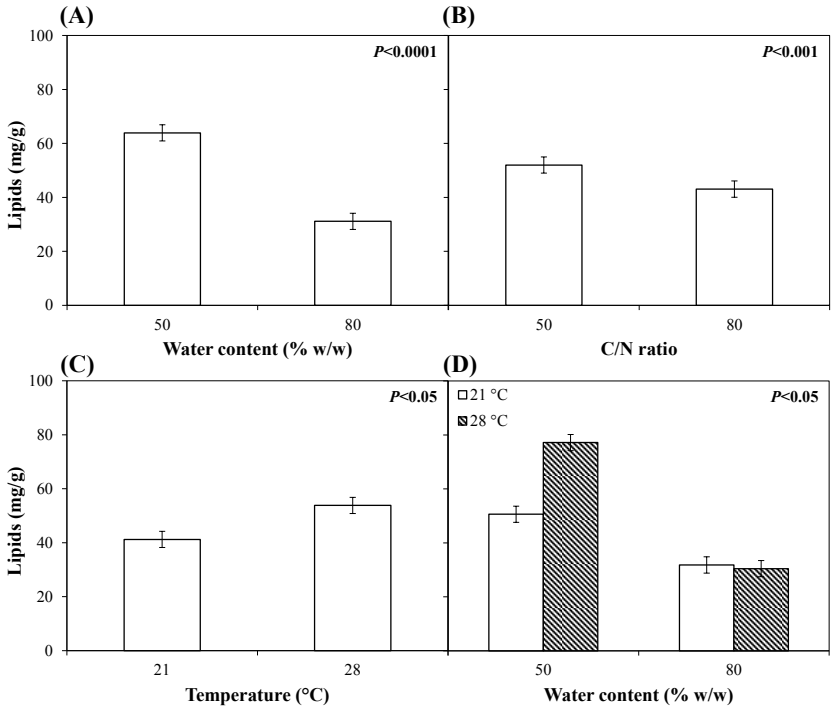

Fig. 3. Main effects of the factors water content (A), C/N ratio (B), temperature (C) on the total lipid accumulation by *Penicillium decumbens* strain M11 and the factor interaction between water content and temperature (D).

P. decumbens M11 failed to grow. The optimal condition for water mass fraction in SSF depends on the microorganism genetics and substrate. For other fungal metabolites obtained in SSF such as enzymes and citric acid, the optimal water fraction levels have been reported to range from 55% to 65% (Balkan and Ertan, 2007; Narayanamurthy *et al*., 2008; El-Gindy *et al*., 2009).

The C/N ratio had a significant effect on the total lipid production from seaweed bagasse by *P. decumbens* M11 ($p < 0.01$). Figure 3B shows the main effect of C/N ratio on lipid accumulation, where on average the total lipids were up to 50 mg/g at a C/N ratio of 50 whereas the total lipid production decreased by 25% at a C/N ratio of 80. Nitrogen limitation is identified as the main nutritional condition that controls lipid accumulation in oleaginous microorganisms (Thevenieau and Nicaud, 2013). This nutritional parameter has mainly been established for oleaginous yeasts with a C/N ratio from 25 to 80, not for filamentous fungi producing lipids from solid substrates (Immelman *et al*., 1997). In this study, the highest lipid content of 96.7 mg/g was attained at a C/N ratio of 50.

The incubation temperature significantly influenced lipid accumulation in *P. decumbens* M11 (p <0.05). Figure 3C displays the main effect of incubation temperature on total lipid accumulation, where on average 40 mg/g were produced at 21°C, increasing by 25% at 28°C. Temperature governs the fungal growth, lipid accumulation and fatty acid profiles in microorganisms (Thevenieau and Nicaud, 2013). Mycelial growth and lipid content increase as the temperature increases, and the optimal temperature depends on the microorganism. For some species of *Penicillium*, the growth rates increase with the temperature (Suutari, 1995). This pattern is common in oleaginous fungi such as *Cunninghamella* and *Mortierella*, in which the biomass and lipid yield improve at higher temperatures and peak at approximately 20°C (Lindberg and Molin, 1993; Conti *et al*., 2001; Dyal *et al*., 2005; Jang *et al*., 2005). Finally, the highest temperature had a significant interaction with the lowest water content improving the lipid accumulation to more than 80 mg/g (p <0.05, Figure 3D).

P. decumbens M11, isolated from estuarine sediments, accumulated 6.4 mg of total lipids per gram of dry seaweed bagasse (6.4 mg/g). When the temperature, water fraction, and C/N ratio were adjusted at 28°C, 50% and 50, respectively, the total lipid production increased to 96.7 mg/g. Table 3 displays a compilation of representative studies that focus on producing lipids from solid substrates. *P. decumbens* and *A. tubingensis* head the list of largest producers of lipids from cellulosic substrates. Thus, our results demonstrate that there is great potential for the *Penicillium* genus in simultaneously performing polysaccharide hydrolysis and lipid synthesis.

Table 3. Total lipid synthesis by different fungal strains grown on solid substrates.

Strains	**Substrate**	**Operation information**	**Lipid accumulation (mg/g)**	**Ref.**
Penicillium decumbens	Seaweed solid wastes	50% of water content; 50 of C/N ratio; 28°C; batch mode	97	This study
Mucor circinelloides	Avicel cellulose	Submerged culture; 28ºC; batch mode	26	Wei et al., 2013
Aspergillus tubingensis	Alkaline-pretreated palm pressed fiber with palm kernel cake	65% of water content; 28°C; batch mode	89	Kitcha and Cheirsilp, 2014
Aspergillus tubingensis	Alkaline pretreated palm empty fruit bunches	65% of water content; 28ºC; batch mode	40	Cheirsilp and Kitcha, 2015
Microsphaeropsis sp	Steam-exploded wheat straw mixed with wheat bran	67% of water content; 30ºC; batch mode	42	Peng and Chen, 2008

Table 4. Profile of fatty acids produced from seaweed bagasse by *Penicillium decumbens* M11.

Factors			Fatty acids (% from extracted lipids)								
C/N	Water fraction (%)	Temp. (°C)	Myristic (C14:0)	Myristoleic (C14:1)	Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Arachidic (C20:0)	Behenic (C22:0)
50	50	21	6.2	1.3	43.5	0.8	6.1	18.9	20.3	2.2	0.6
80	50	21	3.5	0	51.6	1.2	8.1	19.8	15.2	0.5	0.1
50	80	21	2.4	0.2	50.7	1.1	5.4	16.4	23.5	0.1	0.2
80	80	21	2.2	0.3	53.4	0.9	8.3	18.2	14.9	0.9	0.9
50	50	28	1.1	0	44.7	0.3	15.8	20.3	16.7	0.5	0.6
80	50	28	2.4	0	60.7	0.2	19.9	8.4	6.6	0.8	1
50	80	28	1.7	0.3	52.8	0.9	6.5	18.9	15.3	0.6	3.1
80	80	28	1.7	0.1	75.5	0.1	20.9	0.1	0.3	0.1	1.1

Note: standard deviations were lower than 19%.

Table 5. Statistical analysis of factorial design with three variables for saturated lipids in *Penicillium decumbens* strain M11 grown on seaweed solid waste.

Significant variables	Sum mean values	Degree of freedom (df)	Mean square	F-value	Probability (P)
Model	1405	7	351	16.04	< 0.05
A. C/N ratio	592	1	592	27.02	< 0.05
B. Water content	43	1	43	1.97	0.25
C. Temperature	522	1	522	23.82	< 0.05
Interaction BC	249	1	249	11.35	< 0.05
Error	66	8	22		
Total	1470	15			

3.4 Fatty acid profile

Table 4 shows the fatty acid profile of lipids produced from seaweed bagasse by *P. decumbens* M11. The effects of factors on the accumulation of saturated and unsaturated lipids were analyzed as a total. That is, unsaturated lipids were added and then analyzed together, and the same procedure was applied for saturated lipids. The results of the ANOVA showed that only saturated lipids were statistically affected by the studied factors (p <0.05, Table 5). The saturated lipids were unaffected by the water content (Figure 4A), but a C/N ratio of 80 favored the accumulation of saturated lipids, where palmitic acid constituted more than 70% of the sum of saturated lipids (Figure 4B). On the other hand, the highest content of saturated fatty acids was observed at 28°C (Figure 4C), and the interaction between a C/N ratio of 80 and 28°C favored the increased of saturated lipids, where stearic acids reached until 20% of the total lipids (Table 4, Figure 4D). In these fungal cultures, the percentage of saturated fatty acids always exceeded 50% due to the high proportion of palmitic acid (54% on average). The percentage of palmitic acid rose significantly from 41% at 21°C to a maximum of 76% at 28°C. These results are in agreement with previous studies that demonstrated that low incubation temperatures stimulate polyunsaturated fatty acid production (Lindberg and Molin, 1993; Jang *et al*., 2005). Conti *et al*. (2001) noted that microorganisms desaturated membrane fatty acids to regulate fluidity as an adaptive mechanism to maintain membrane function for optimal metabolism. The most abundant unsaturated fatty acids produced by *P. decumbens* M11 from seaweed bagasse were oleic and linoleic acids.

The hydrocolloid industry discharges millions of tons of solid waste annually that are poorly exploited. This seaweed bagasse can be revalorized for lipid production if adequate microorganisms and operational conditions are implemented. In this study, the lipid content of seaweed bagasse was increased from 0.64% to 9.67% on a dry basis using the filamentous fungus *P. decumbens* M11 generated by direct bioconversion. The lipids that were primarily accumulated by this fungal species were, in descending order, palmitic>stearic>oleic>linoleic acid. These lipids can be used as animal food or for biodiesel fuel. In comparison with another biological options for lipid production, for example by using oleaginous microalgae (Robles-Hereida *et al*., 2016; Navarro-Peraza *et al*., 2017), filamentous fungi use a wide variety of insoluble complex substrates such as lignocellulosic biomasses without pretreatment requirements due to their cellulolytic activity.

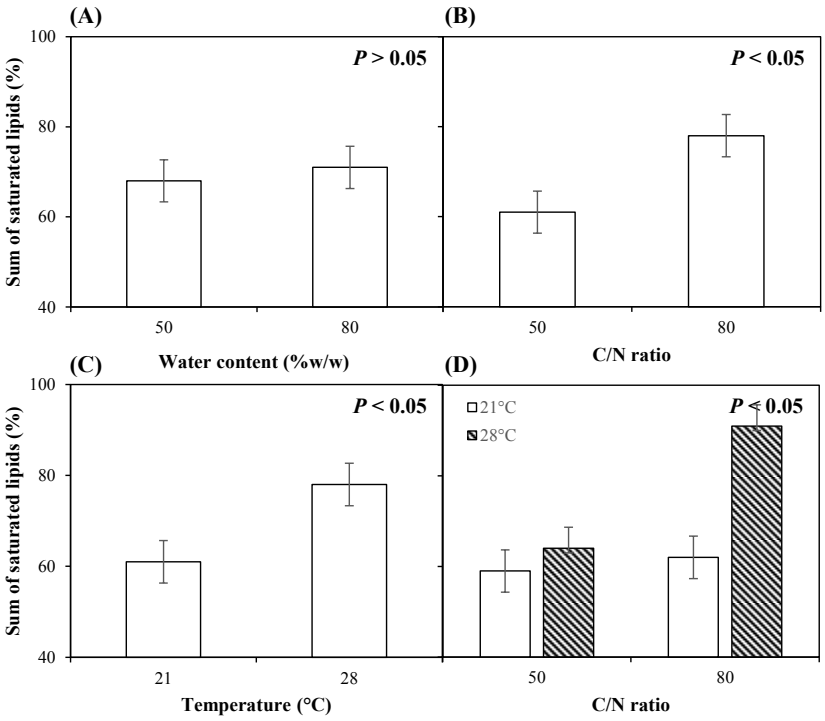

Fig. 4. Main effects of the factors water content (A), C/N ratio (B), temperature (C) on the saturated lipids in *Penicillium decumbens* strain M11 and the factor interaction between C/N ratio and temperature (D).

In spite of this, further studies are needed to increase the potential of lipid accumulation by *Penicillium* spp. by controlling the environmental conditions.

Conclusions

Filamentous ascomycetes fungi are candidates for waste biorefineries because of their hydrolytic capacities and production of a wide range of high-value metabolites. In particular, the *Penicillium* genus mediates several industrial processes, and their capacity to accumulate lipids from soluble substrates has been recognized for decades. In this study, we isolated a fungus identified as *P. decumbens* M11. It grew on a cellulosic substrate, seaweed bagasse, producing nearly 6 mg/g of lipids. When the temperature, nitrogen content, and water fraction were adjusted at 28°C, C/N ratio of 50 and 50%, respectively, the total lipid production from algal carbohydrates increased 15 times. The incubation temperature modulated the fatty acid profile although palmitic, oleic, and linoleic acids were predominant for all treatments. This exploratory study demonstrated that *Penicillium* has the same potential as a lipid producer as other filamentous ascomycetes fungi such as *Aspergillus*, thus encouraging further in-depth research.

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