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LA COLECCIÓN DE HONGOS ENDÓFITOS QUITO CATÓLICA (CEQCA): EVALUACIÓN DE LA CAPACIDAD DE DEGRADACIÓN DE POLIÉSTER POLIURETANO

Disertación previa a la obtención del título de Licenciada en Ciencias Biológicas

DIANA CAROLINA BAUTISTA BARRIONUEVO

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Certifico que la Disertación de Licenciatura en Ciencias Biológicas de la Srta. Diana Carolina Bautista Barrionuevo ha sido concluida de conformidad con las normas establecidas; por lo tanto, puede ser presentada para la calificación correspondiente.

M.Sc. Alexandra Narváez Trujillo

Directora de la Disertación

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"El milagro que adivinó tu anhelo, con su magia te acercó hasta aquí. Ya comienzan a bajar del cielo las palabras que hablarán por ti"

Antonio Mateo Allende

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"La gratitud es la memoria del corazón" Joseph Wood Krutch

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LISTA DE ABREVIATURAS

Abreviatura	Significado
ANOVA	Analysis of Variance
°C	Celsius degrees
CEQCA	Colección de Hongos Endófitos Quito Católica
DLN	Anionic Aliphatic Polyester Polyurethane dispersion
FT IR	Fourier Transformed Infra-Red
NMDS	Non-Metric Multidimensional Scaling
PAST	PAleontological STatistics
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PUR	Polyester Polyurethane
PUR-A	Polyester Polyurethane solid
PUR-L	Polyester Polyurethane liquid
Rpm	Revolutions per minute
SCSA	Sole Carbon Source Assay
SPSS	Statistical package of Social Science

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RESUMEN

La contaminación ambiental es el mayor problema para la humanidad puesto que representa el uso indiscriminado de los recursos naturales y el deficiente manejo de los desechos generados. Particularmente el plástico es uno de los materiales sintéticos que puede tardar hasta 1000 años en degradarse; entre los polímeros plásticos más importantes está el poliéster poliuretano (PUR) que puede ser degradado por microorganismos. El presente estudio evalúa la capacidad de degradación de PUR de cincuenta y ocho hongos endófitos de la Colección de Hongos Endófitos Quito Católica (CEQCA) y cuatro hongos endosimbiontes de escarabajos. Trece hongos endófitos y dos hongos endosimbiontes presentan capacidad de degradación de PUR y una actividad importante fue observada en siete hongos: *Fusarium equiseti* (CEQCA M1324), CEQCA O3215, *Clonostachys/Bionectria rossmaniae* (CEQCA O3783), *Plectosphaerella cucumerina* (CEQCA M1268), *Pestalotiopsis mangiferae* (CEQCA O3060), *Pestalotiopsis* sp. (CEQCA O3064) y *Hypoxylon investiens* (CEQCA I1349). Estos hongos degradan 100% de PUR entre el tercer y séptimo día de crecimiento en este medio. Adicionalmente, los siete hongos son capaces de degradar PUR en un ambiente anaerobio usando Impranil DLN como la única fuente de carbono. La degradación de PUR determinada por la absorbancia en luz visible y la disminución de vibraciones moleculares comprobada por espectroscopia infrarroja con transformadas de Fourier sugiere una actividad enzimática. Este es el primer reporte de la capacidad de degradación de PUR para *Fusarium equiseti* (CEQCA M1324), *Clonostachys/Bionectria rossmaniae* (CEQCA O3783), *Plectosphaerella cucumerina* (CEQCA M1268), *Pestalotiopsis mangiferae* (CEQCA O3060) y *Hypoxylon investiens* (CEQCA I1349).

Palabras clave: CEQCA, Impranil DLN, Hongos Endófitos, PUR

ABSTRACT

Environmental contamination is the most serious problem for humanity since it represents the indiscriminate use of natural resources and poor waste management. Particularly, plastic is one of the synthetic materials which can take up 1000 years to degrade. Among the most important plastic polymers is polyester polyurethane (PUR) that can be degraded by microorganisms. The present study evaluate the PUR degradation ability of fifty eight endophyte fungi catalogued in the Quito Católica Endophyte Fungi Collection "CEQCA" and four beetle endosymbiont fungi. Thirteen endophytic fungi and two endosymbiont fungi exhibited the ability to degrade PUR and important activity was observed in seven fungi: *Fusarium equiseti* (CEQCA M1324), CEQCA O3215, *Clonostachys/Bionectria rossmaniae* (CEQCA O3783), *Plectosphaerella cucumerina* (CEQCA M1268), *Pestalotiopsis mangiferae* (CEQCA O3060), *Pestalotiopsis* sp. (CEQCA O3064) and *Hypoxylon investiens* (CEQCA I1349). These fungi degraded 100% of PUR between the 3rd and 7th day of growth in this media. Additionally, these seven fungi are able to degrade PUR in an anaerobic environment using Impranil DLN as the sole carbon source. The PUR degradation determined by the absorbance in visible light and the reduction of molecular vibes proven by Fourier transformed infrared spectroscopy suggest an enzymatic activity. This is the first report of PUR degradation ability to *Fusarium equiseti* (CEQCA M1324), *Clonostachys/Bionectria rossmaniae* (CEQCA O3783), *Plectosphaerella cucumerina* (CEQCA M1268), *Pestalotiopsis mangiferae* (CEQCA O3060) and *Hypoxylon investiens* (CEQCA I1349).

Key words: CEQCA, Endophyte fungi, Impranil DLN, PUR

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REVISTA

Environmental Science and Pollution Research

TÍTULO

Quito Católica Endophyte Fungi Collection "CEQCA": Evaluation of polyester polyurethane degradation ability

AUTORES

Diana Bautista, Alexandra Narváez*

Laboratorio de Biotecnología Vegetal, Escuela de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador.

D. Bautista: A. Narváez (*)

Laboratorio de Biotecnología Vegetal, Escuela de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Pontificia Universidad Católica del Ecuador, código postal, Quito, Ecuador.

E-mail: anarvaez@puce.edu.ec

Tel. +593 2 2991700 ext. 1810

Fax. +593 2 2991687

Abstract

Environmental contamination is the most serious problem for humanity since it represents the indiscriminate use of natural resources and poor waste management. Particularly, plastic is one of the synthetic materials which can take up 1000 years to degrade. Among the most important plastic polymers is polyester polyurethane (PUR) that can be degraded by microorganisms. The present study evaluate the PUR degradation ability of fifty eight endophyte fungi catalogued in the Quito Católica Endophyte Fungi Collection "CEQCA" and four beetle endosymbiont fungi. Thirteen endophytic fungi and two endosymbiont fungi exhibited the ability to degrade PUR and important activity was observed in seven fungi: *Fusarium equiseti* (CEQCA M1324), CEQCA O3215, *Clonostachys/Bionectria rossmaniae* (CEQCA O3783), *Plectosphaerella cucumerina* (CEQCA M1268), *Pestalotiopsis mangiferae* (CEQCA O3060), *Pestalotiopsis* sp. (CEQCA O3064) and *Hypoxylon investiens* (CEQCA I1349). These fungi degraded 100% of PUR between the 3rd and 7th day of growth in this media. Additionally, these seven fungi are able to degrade PUR in an anaerobic environment using Impranil DLN as the sole carbon source. The PUR degradation determined by the absorbance in visible light and the reduction of molecular vibes proven by Fourier transformed infrared spectroscopy suggest an enzymatic activity. This is the first report of PUR degradation ability to *Fusarium equiseti* (CEQCA M1324), *Clonostachys/Bionectria rossmaniae* (CEQCA O3783), *Plectosphaerella cucumerina* (CEQCA M1268), *Pestalotiopsis mangiferae* (CEQCA O3060) and *Hypoxylon investiens* (CEQCA I1349).

Key words: CEQCA, Endophyte fungi, Impranil DLN, PUR

Introduction

Environmental contamination is one of humanity´s major problems. Environmental contamination represents both, the unreasonable use of natural resources and the deficient management of generated waste. It is known that approximately 140 million tons of synthetic polymers are produced every year around the world (Shimao 2001), of which, only 14% is recycled or reused in certain countries (De Coverly et al. 2008). Plastic, in particular, is one of the world´s most used synthetic polymers that can take up to 1000 years to degrade.

One of the most important plastic polymers is polyurethane. Polyurethane uses range from medical (Darby &Kaplan 1968, Howard et al. 2012, Zafar et al. 2013) to industrial applications as in plastic foam manufacturing, cushions, synthetic leathers, adhesives, paints and fibers (Nakajima-Kambe et al. 1999, Tokiwa et al. 2009). In the year 2000, polyurethane global consumption was close to 8 million tons. For 2016, it is expected to grow up to 9.6 million tons (Peng et al. 2014), an alarming increase over time.

The advantage of polyester polyurethane plastic (PUR) above others is that the urethane compounds can be hydrolyzed by microorganisms in two processes: Urethane bonds degradation and polyol segment degeneration (Nakajima-Kambe et al. 1999), the hydrolysis is catalyzed by microorganism-produced extracellular enzymes like esterases, proteases, ureases and polyurethanases (Pathirana &Seal 1984).

Russell et al. (2011) were pioneers in the exploration of PUR degradation by endophyte fungi. Endophyte fungi are a hyperdiverse group that live in plant tissues without producing evident symptoms of pathology in the host, and are known to establish complex symbiotic relations (Rodriguez et al. 2009). Endophyte fungi play an important role by increasing heavy metal tolerance and drought resistance, reducing herbivore attacks, increasing pathogen defense and improving growth and competition ability (Van Bael et al. 2005); they also contribute to lignocellulose polymer degradation (Russell et al. 2011).

Through research, many fungi capable of PUR degradation have been found: *Alternaria, Aspergillus niger, A. flavus, A. fumigatus, A. versicolor, Aureobasidium pullulans, Chaetomium globosum, Cladosporium, Curvularia senegalensis, Emericella, Fusarium solani, Geomyces, Gliocladium roseum, Nectria, Neonectria, Penicillium citrinum, P. funiculosum, Pestalotiopsis microspora, Phoma, Plectosphaerella cucumerina and Trichoderma* (Barratt et al. 2003, Bentham et al. 1987, Cosgrove et al. 2007, Nakajima-Kambe et al. 1999, Russell et al. 2011). A strain of *Pestalotiopsis microspora,* an endophyte fungus collected in Yasuní Rainforest in Ecuador, was described by Russell et al. (2011) as a microorganism

capable of PUR degradation in aerobic and anaerobic environments. This Ecuadorian strain of *P. microspora* (CEQCA O1162) has the maximum PUR degradation ability in aerobic environments when compared to the other fungi, and, when tested in anaerobic environments, the strain shows the same level of degradation than in aerobic environments.

Russell et al. (2011) evaluated several fungi, of which the strain of *P. microspora* (CEQCA O1162) had a robust PUR degradation activity in aerobic conditions which was conserved in anaerobic environment. *P. microspora* (CEQCA O1162) is a cosmopolite species that has been reported as having capacities like taxol production, but up to date only the Ecuadorian strain of *P. microspora* (CEQCA O1162) has demonstrated PUR degradation ability. This strain of *P. microspora* (CEQCA O1162) is catalogued in the CEQCA collection. The CEQCA collection has about 5124 accessions of fungi, isolated from countless plant species in various ecosystems in Ecuador and lately includes also endosymbiont fungi isolated from Passalidae beetles. This abundant biodiversity can also imply a large and yet undiscovered chemical reservoir, among which could be bioremediation abilities (Russell et al. 2011), our objective was to evaluate if other species of endophyte fungi, including other *Pestalotiopsis* sp. from the CEQCA collection, exhibited an ability comparable to the *P. microspora* (CEQCA O1162) strain reported by Russell et al. (2011). Results of the author's study contribute to the knowledge of endophyte fungi potential and provide the basis for bioremediation in Ecuador.

Materials and methods

Setting

Quito Católica Endophyte Fungi Collection "CEQCA" has been established from endophyte fungi isolated from many plant species in several ecosystems in Ecuador. Fungi are isolated in culture media, particularly Potato Dextrose Agar (PDA). Identified by molecular methods (ITS1-4 region sequencing) and preserved in barley seeds storing them at -80 ^{o}C.

Fungi selection

Sixty two fungi were selected from the CEQCA collection based on previous reports of microorganisms with PUR degradation ability. Selection aimed at achieving a population that represents: taxonomic diversity, different collection ecosystems, and three growing rates (slow/moderate/fast) according to CEQCA database. A total of sixty two isolates were selected, among fifty eight are endophyte fungi and

four were isolated from the intestines of the wood borer Passalidae beetles. Fungi maintained at -80 °C were recorvered by culturing in PDA from 1 to 2 weeks at 24 °C. Based on Russell et al. (2011) the strain *P. microspora* (CEQCA O1162) from the CEQCA collection, was selected as a positive control for PUR degradation in aerobic and anaerobic environments. Taxonomic identification for the fungi selected had been previously achieved by sequency of the ITS region (White et al. 1990), and this information was recovered from the CEQCA database.

PUR degradation

The methodology for *in vitro* PUR degradation at 24 °C by Russell et al. (2011) was followed in order to compare our results to the PUR degradation ability reported by these authors. After 2 weeks of fungi culture growth in PDA, a plug of 0.5 cm^3 of agar (with active growing fungi) was used for each of the following PUR assays. Polyester polyurethane (PUR): Impranil DLN is a milky suspension that becomes transparent upon degradation (Peng et al. 2014). Organisms capable of degrading this polymer cause a turbidity change from opaque to translucent (Russell et al. 2011) .

PUR-A assay

Fungi cultures were transferred to a petri dish with culture media containing: 19 mM NaH2PO4, 33.5 mM K2HPO4, 7.6 mM (NH4)2 SO4; 2.5 mM Na citrate, 250 µM MgSO4 19 µM thiamine, 0.05% casamine acid, 147 µM FeCl3 $_+$ 6 H_2O, 14 µM $ZnCl_2$ $_+$ 4 H_2O, 12 µM $CoCl_{2+}$ 6 H_2O, 12 µM Na_2MoO_{4+} 2 H_2O, 10 µM $CaCl_{2+}$ 2H_2O, 11 µM $CuCl_2$, 12 µM $MnCl_2$, 12 µM H_3BO_3 y 1.8 mM HC, 15 ml agar and 1 ml Impranil DLN an anionic aliphatic aqueous PUR dispersion (Bayer Material Science) in 1 liter of solution.

Fungi with PUR degradation ability displayed clearance zones around the growing culture (Russell et al. 2011). This method is widely used in the detection of microorganisms able to degrade polymers by means of production of extracellular enzymes that diffuse through agar (Crabbe et al. 1994).

PUR-L clearance assay

PDA plugs of fungi cultures that exhibited positive activity in the PUR-A assay were transferred to an assay tube containing 5 ml of liquid media of Impranil DLN (following the same recipe as for the solid media without agar). A 250 µl sample was taken and centrifuged at 1310 rpm for 2 min, absorbance of the liquid fraction was measured at 595 nm using Eppendorff Bio-photometer Plus. PUR-L degradation was defined

by turbidity change from opaque to translucent. Measures were taken every day for two weeks, in triplicate, sterile water was used as blank and PUR-L without fungus as a negative control (Gautam et al. 2007). Absorbance was interpreted as the percentage of media clearance.

Sole carbon source assay

Fungi that showed PUR-degradation activity were tested for their ability to use PUR as a sole carbon source in a triplicate assay by using Impranil DLN (not containing N methyl pyrrolidone). Fungi cultures were grown in potato dextrose broth (PDB) for 1 week. Cultures were then homogenized by vigorous shaking and 1 ml of each culture was centrifuged at 1310 rpm for 2 min. The supernatant was removed and the fungal pellet was re-suspended in 1 ml of PUR-L media. Samples were centrifuged and re-suspended again. The 1ml of the re-suspended sample (washed fungal inoculums) was added to sterile culture tubes to a final volume of 5 ml PUR-L. The cultures were then monitored for PUR-L degradation as a sole carbon source. Samples were measured every 48 hours for a total of 2 weeks using an Eppendorff Bio-photometer Plus at 595 nm.

Anaerobic degradation of PUR

Culture tubes containing 4 ml of PUR-L media and 1ml of washed fungal inoculums were incubated under anaerobic environments in a quadruplicate assay. An anaerobic environment was generated using a 2.5 liters sealed container with a CO_2Gen sachet (Oxoid). For each fungus, three sample tubes were placed in the anaerobic environment and 1 tube in the aerobic environment. Samples were measured at 7 and 15 days at 595 nm using an Eppendorff Bio-photometer Plus.

FT IR analysis of PUR degradation

The infrared (IR) spectras were measured with FT IR Spectra BX Perkin Elmer after two weeks of fungi growth in PUR-L media compared with PUR-L media spectra and the media without PUR-L spectra, the background spectra corresponds to chlorotrifluoroethylene (solvent without carbon- carbon bonds).

Absorbance measures in PUR-L

At the end of the PUR-A, PUR-L, Sole carbon source and anaerobic assays, control tubes without fungi were centrifuged at 1310 rpm per 2 min. Supernatant was taken for a dilution assay as follows: 1/1, 1/2,

1/5, 1/10, and 1/20. Absorbance measures were taken at 595 nm in Spectronic Genesis 2 to determine degradation percentages. The clearance percentages obtained from the absorbance measurements of triplicate sample´s average of the last day compared with the PUR-L dilution, the obtained results correspond to PUR concentration that inversely proportional to clearance percentages.

Data Analysis

Analysis of variance (ANOVA) was performed with the Statistical Package of Social Science (SPSS) V.19. Using *P. microspora* (CEQCA O1162) as a positive control and PUR-L media as a negative control an ANOVA was performed on the absorbance measures of the assayed fungi. With the ANOVA data, Duncan´s test for statistical significance was applied to categorize the observed degradation abilities of the assayed fungi into ranges.

Results and discussion

PUR degradation

Endophyte fungi are a promising biological source of biomolecules for bioremediation. Russell et al. (2011) were the pioneers of endophyte fungi studies on plastic polymer degradation. By using *P. microspora* (CEQCA O1162) as a positive control and by following the methodology described in Russell et al. (2011) results from our study can be directly compared to the report of the PUR degradation activity in *P. microspora* (CEQCA O1162). This is especially important given that this endophyte strain exhibits a robust in anaerobic conditions, a characteristic that is useful when considering potential use in landfill conditions. In this study, fifty eight endophyte fungi isolated within the Ascomyota division and four endosymbiont fungi isolated from Passalidae beetle were selected from the CEQCA which houses a representative collection of Ecuadorian endophyte fungi. The fungi selected for this study belong to orders with previous reports for PUR degradation, Xylariales, Hyocreales, Phyllachorales, and Pleosporales. In these orders, genres with previous reports of biodegradation activity were ***Alternaria, Cladosporium, Fusarium, Gliocladium, Penicilium, Pestaloptiosis, Plectosphaerella, Trichoderma and Xylaria.*** **There have been no reports of biodegradation activity for the genus *Albonectria, Archersonia, Bionectria, Colletotrichum, Entonaema, Hypoxylon, Humicola, Mazzantia, Monochaetria, Periconia, Phialemonium, Phomopsis, Preussia, Podospora.and Sydowiellia.***

PUR-A assay

A collection of sixty two fungi isolates were screened for their ability to degrade PUR-A following Crabbe et al. (1994) methodology with a PUR-A assay as the initial screen. Thirty nine endophyte fungi and two endosymbiont fungi produced a halo of clearance indicating PUR-A degradation (Fig. 1)) Of these, *Sydowiella* (CEQCA M1302) and *Fusarium solani* (CEQCA P0484) produced a halo at 7 days which is comparable to the *P. microspora* (CEQCA O1162) positive control. PUR degradation was performed with production of extracellular, secreted, and diffusible enzymes, probably some type of polyurethanase (Crabbe et al. 1994). Since all of the fungi able to degrade PUR grew during the degradation process, the degradation by-products are not toxic for the survival and growth of these degraders (Peng et al. 2014).

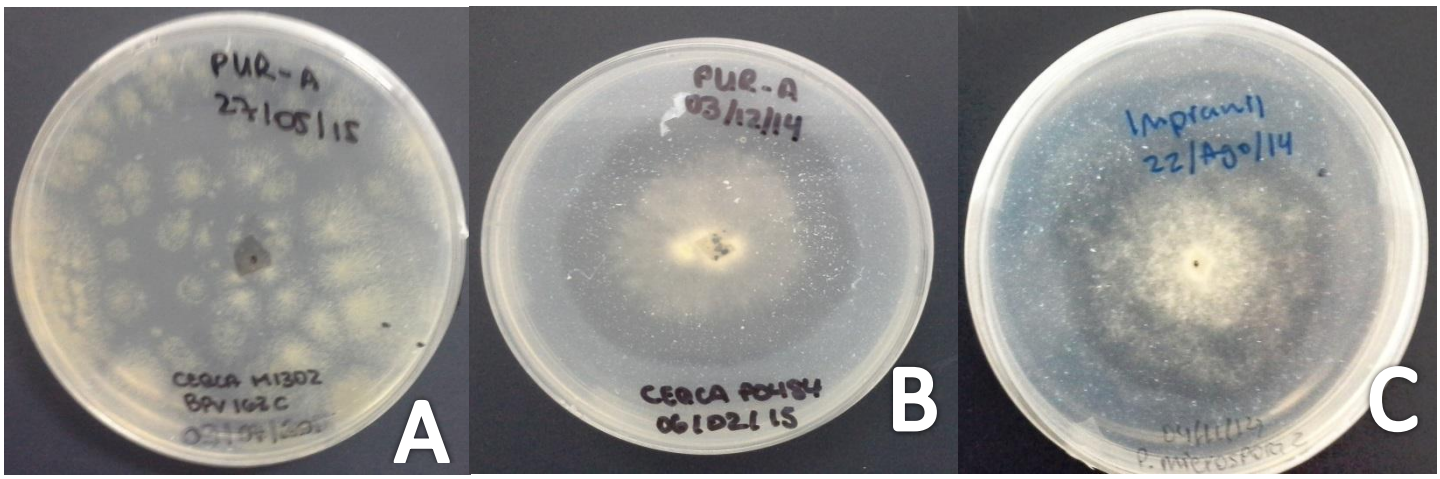

Fig. 1 Example of PUR-A screen for PUR clearance after 1 week of fungal growth. (A) *Sydowiella* (CEQCA M1302) (B) *Fusarium solani* (CEQCA P0484) (C) *Pestalotiopsis microspora* (CEQCA O1162) positive control

PUR-L clearance assay

A collection of thirty nine isolated fungi were screened for their ability to grow on and degrade PUR-L. Of the thirteen endophyte and two endosymbiont fungi that degraded more than 50% of PUR-L, three showed a robust degradation activity (100%), five exhibited a degradation activity between 80% and 100%, including positive control *P. microspora* (CEQCA O1162) while seven showed moderate activity between 60% and 80% (Table 1). PUR-L degradation was evidence by a turbidity change (Fig. 2) from opaque to translucent in PUR-L media (Russell et al. 2011). Degradation kinetics of the fifteen species was performed at 24°C. These isolated fungi showed the highest degradation efficiency between the 3rd and 7th day of the assay.

Table 1 Endophyte fungi with clearance percentage of PUR-L determined by absorbance measures.

CEQCA CODE	Endophyte	Clearance percentage
CEQCA-I1349	*Hypoxylon investiens*	76,7%
CEQCA-M1268	*Plectosphaerella cucumerina*	100%
CEQCA-M1322	*Cladosporium cladosporioides*	61%
CEQCA-M1324	*Fusarium equiseti*	62%
CEQCA-M1326	*Alternaria arborescens*	77,2%
CEQCA-O3059	*Colletotrichum gloeosporioides*	76,6%
CEQCA-O3060	*Pestalotiopsis mangiferae*	88%
CEQCA-O3064	*Pestalotiopsis* sp.	97%
CEQCA-O3215	Unidentified	89%
CEQCA-O3783[b]	*Clonostachys/Bionectria rossmaniae*	90,3%
CEQCA-O4962[b]	*Trichoderma spirale*	75,9%
CEQCA-P0484	*Fusarium solani*	100%
CEQCA-P0501	*Gliocladium* sp.	100%
CEQCA-P2406	*Alternaria* sp.	66,7%
CEQCA-O1162[a]	*Pestalotiopsis microspore*	88,7%

[a] Positive control

[b] Endosymbiont fungi

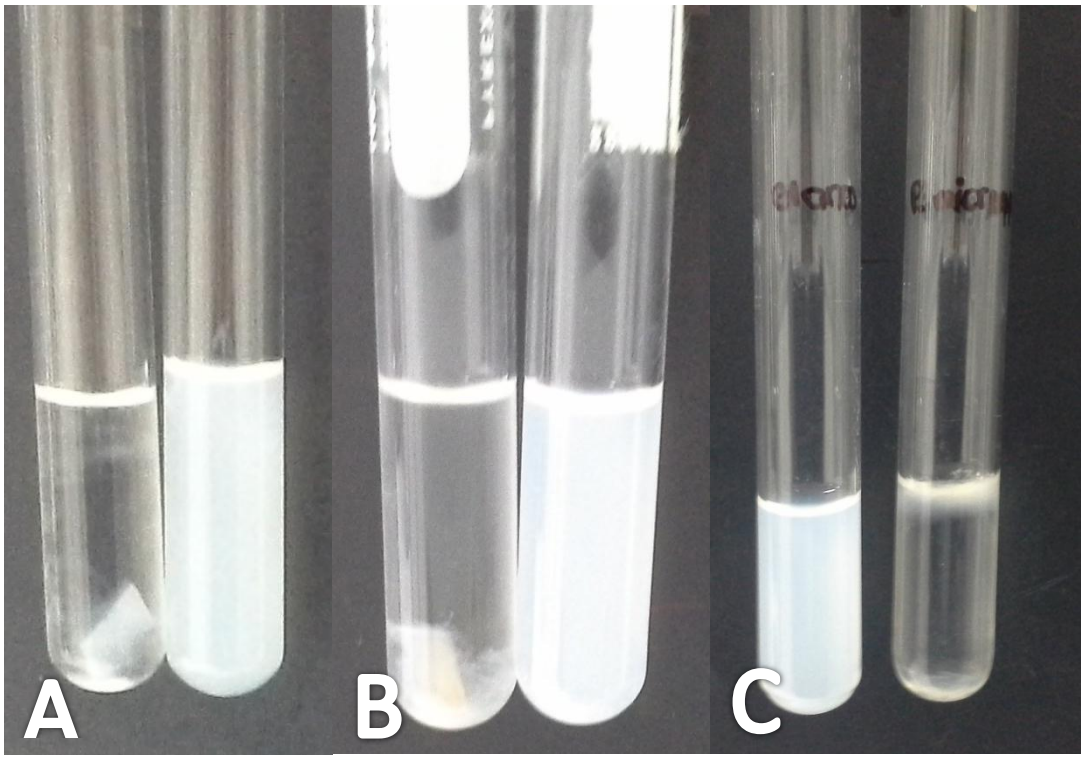

Fig. 2 Examples of PUR-L cultures at 2 weeks after inoculation, (A) *Plectosphaerella cucumerina* (CEQCA M1268), (B) *Hypoxylon investiens* (CEQCA I1349), (C) *Pestalotiopsis microspora* (CEQCA O1162) positive control.

Sole carbon source assay and anaerobic degradation of PUR

Endophyte fungi that showed high activity in PUR-L assay were screened for their ability to use PUR as a sole carbon source in aerobic and anaerobic environments (Fig. 4). Seven endophyte fungi were able to degrade PUR with Impranil DLN serving as a sole carbon source in both environments (60% - 100%) (Table 2). For *Plectosphaerella cucumerina* (CEQCA M1268), *Fusarium equiseti* (CEQCA M1324), *Pestalotiopsis mangiferae* (CEQCA O3060) and *Pestalotopsis* sp. (CEQCA O3064) degradation ability was conserved and not significantly different when fungi were grown under aerobic or anaerobic environments as was reported by Russell et al. (2011) for positive control *P. microspora* while *Hypoxylon investiens* (CEQCA I1349) and *Clonostachys/Bionectria rossmaniae* (CEQCA O3783) present major degradation in aerobic environment and CEQCA O3215 degraded more in anaerobic environment (Fig. 3). However, we observed during this research that some isolates at times lose this ability and renewal of the fungal isolate from its initial stock is necessary to recover the degradation ability. This is particularly applicable to the positive control used in this study, *P. microspora* (CEQCA O1162). For this reason the positive control was excluded in this assay, however this type of observation has a practical significance for fungal growth and on metabolism of PUR that could be used in anaerobic fermentation systems (Peng et al. 2014).

Table 2 Fungi isolates with clearance percentage in sole carbon source assay (SCSA) and anaerobic environment assay

CEQCA CODE	Endophyte	Clearance percetage SCSA	Anaerobic clearance percentage
CEQCA-I1349	*Hypoxylon investiens*	98,7%	86,7%
CEQCA-M1268	*Plectosphaerella cucumerina*	99,7%	95,7%
CEQCA-M1324	*Fusarium equiseti*	76,7%	77,1%
CEQCA-O3060	*Pestalotiosis mangiferae*	80%	87,6%
CEQCA-O3064	*Pestalotiopsis* sp.	61,8%	94,3%
CEQCA-O3215	Unidentified	94,6%	90%
CEQCA-O3783	*Clonostachys/Bionectria rossmaniae*	60,6%	73,2%
CEQCA-P0484	*Fusarium solani*	N/D	N/D
CEQCA-O1162	*Pestalotiopsis microspora*	0%	0%

[a], N/D stands for non-determined due to contamination

[b] Positive control

[c] Endosymbiont fungi

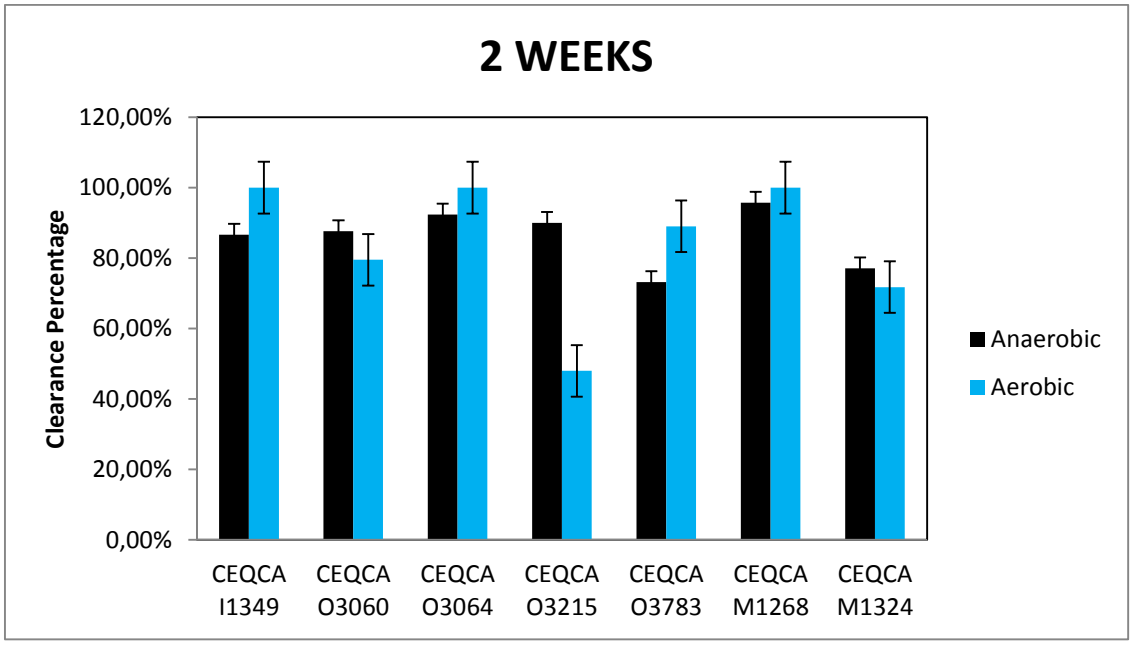

Fig. 3 Comparison of Endophyte fungi between anaerobic and aerobic environments with standard error bars

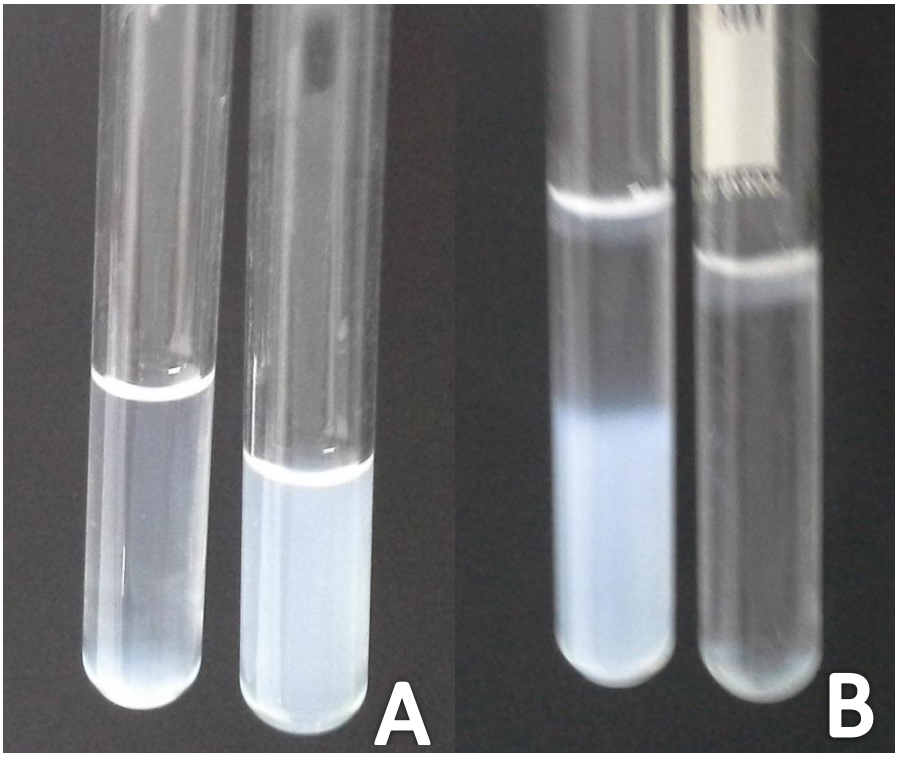

Fig. 4 Examples of PUR-L cultures at two weeks after inoculation, (A) CEQCA O3215 as sole carbon source assay, (B) Plectosphaerella cucumerina (CEQCA M1268) in anaerobic degradation vs. aerobic control

FT IR analysis of PUR degradation

The mechanism of PUR-L degradation was investigated by Infrared spectroscopy (Russell et al. 2011). PUR-L media sample displays molecular vibes between 3500 and 4387,7 cm^{-1} with an approximate absorbance of 1,5 nm, while the fungus *Cladosporium cladosporioides* (CEQCA M1322) generated molecular vibes near to 0 nm of absorbance as well as the media without PUR-L (Fig. 5).

This shows that the fungus reduces the molecular vibes for the hydrolysis the PUR-L media´s functional groups. The reduction of molecular vibes is consistent with the turbidity change from opaque to translucent. FT-IR evaluation of the other putatively degrading fungi must be evaluated, especially in the case of *Plectosphaerella cucumerina* (CEQCA M1268) which exhibited the major clearance of the media.

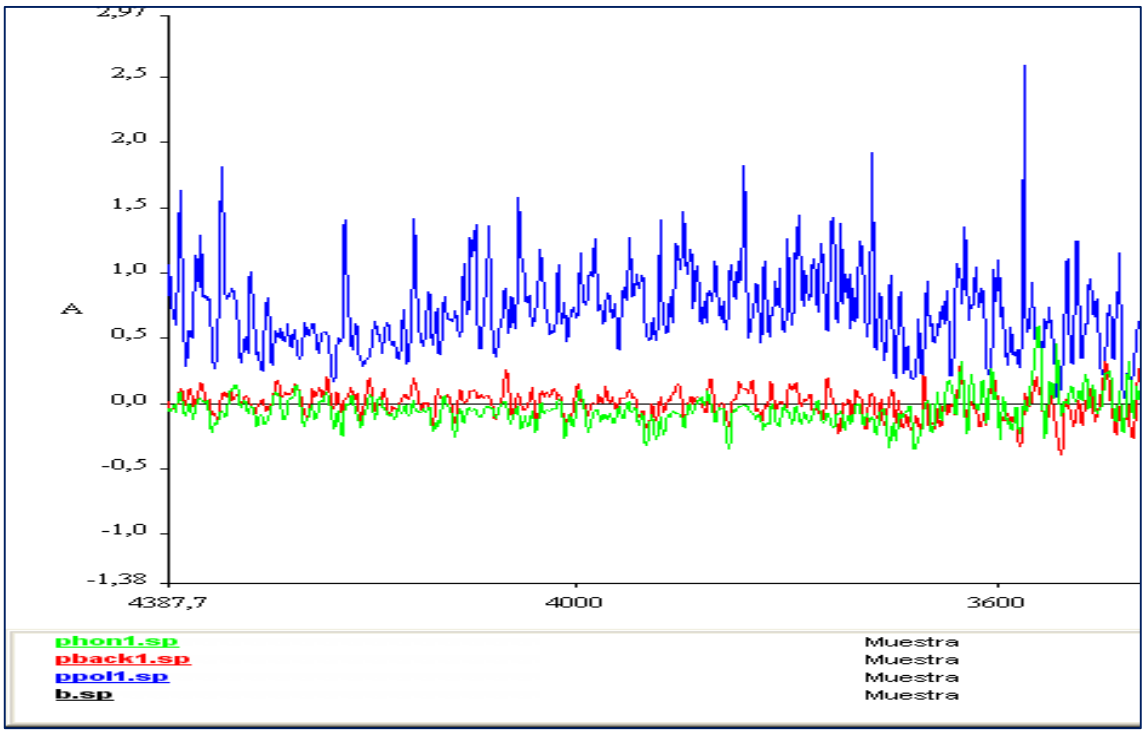

Fig. 5 Infrared spectra: black line represents background, red vibes correspond to mediun without PUR, the blue vibes belong to PUR-L media and the green vibes correspond to Cladosporium cladosporioides (CEQCA M1322) after two weeks of growth

All active fungi were identified as Sordariomycetes of the Ascomycota division. Three orders of endophytic fungi were evaluated for their PUR degradation ability: Hypocreales, Phyllochorales and Xylariales. Within the order Hypocreales *Fusarium equiseti* (CEQCA M1324) was able to use PUR as a sole carbon source in aerobic and anaerobic conditions. Bentham et al. (1987) reported *Fusarium* as a fungus responsible for the degradation of soil-buried polyester polyurethane and Crabbe et al. (1994) recovered *Fusarium solani* in

soil polyurethane cultures, these are the only reports regarding the PUR degradation ability of *Fusarium.* The isolate CEQCA O3215 has a 94% degradation percentage in the sole carbon source assay and maintains this degradation ability in an anaerobic environment. Taxonomic identification of this endophyte was not possible, sequence similarity and phylogenetic approaches classify it as belonging to the Hypocreales order, and therefore could represent a new species. *Clonostachys/Bionectria rossmaniae* (CEQCA O3783) is the only endosymbiont fungus isolate with an ability to use PUR as a sole carbon source and in an anaerobic environment. This is the first report for this type of bioactivity for this genus. Interestingly, this endosymbiont fungus was isolated from the intestine of a wood boring beetle of the Passalidae family and opens the way to questions relating the complex interactions betweeen endophytic fungus and their host plants as insect endosymbionts.

Plectosphaerella cucumerina (CEQCA M1268) of the Phyllochorales order is the most robust PUR degrader identified in this study. It exhibited a 99, 7% clearance in the sole carbon source assay and retained 95, 7% degradation in an anaerobic environment. The Duncan´s test revealed that *Plectosphaerella cucumerina* (CEQCA M1268) is the best degrader in the PUR-L assay. *Plectosphaerella cucumerina* was reported by Cosgrove et al. (2007) in acid soil with polyester polyurethane compounds; its frequency was <5% of the colonies and didn´t produce clearance zones in Impranil agar, therefore this study is the first report for *Plectosphaerella cucumerina* as a degrader of PUR.

The order Xylariales has three fungi isolates with PUR degradation ability, *Pestalotiopsis mangiferae* (CEQCA O3060) and *Pestalotopsis* sp. (CEQCA O3064) both exhibited PUR degradation in anaerobic conditions supporting the potential ability of the genus *Pestalotiopsis* in PUR bioremediation as reported by Russell et al. (2011). *Hypoxylon investiens* (CEQCA I1349) demonstrated a high PUR degradation in SCAS (98, 7%), although in an anaerobic environment it was considerably less 86, 7%. Nevertheless, this is the first report of PUR degradation in. *Hypoxylon investiens.*

Conclusion

This investigation established the robust polyurethane degradation activity by Ecuadorian endophyte fungi: *Fusarium equiseti* (CEQCA M1324), CEQCA O3215, *Clonostachys/Bionectria rossmaniae* (CEQCA O3783), *Plectosphaerella cucumerina* (CEQCA M1268), *Pestalotiopsis mangiferae* (CEQCA O3060),

Pestalotiopsis sp. (CEQCA O3064) and *Hypoxylon investiens* (CEQCA I1349). These seven microorganisms are incredible PUR degraders under aerobic and anaerobic conditions in which the synthetic polymer served as a sole carbon source.

PUR degradation is not an exclusive ability of endophyte fungi and more microorganisms with this ability are expected to be found in the future. However, this work establishes endophyte's potential and usefulness as a biodiversity source for bioremediation applications. A better understanding of the complex metabolism of PUR degradation by different endophyte species would be helpful in applying its characteristics for waste management and material recycling.

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