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Isolation and Screening of Extracellular Lipase-Producing Endophytic Fungi from *Handroanthus impetiginosus*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IMS and GJB performed the experiments under the guidance of authors DBH and JHHL. Author DBH also prepared the manuscript for publication. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This study aimed to screen extracellular lipase-producing endophytic fungi isolated from *Handroanthus impetiginosus* (*H. impetiginosus*).
Study Design: Endophytic fungi were isolated and screened for extracellular lipase production. The best strains obtained were tested for lipase production, via submerged fermentation, using two different carbon sources.
Place and Duration of Study: Institute of Chemistry, Federal University of Alfenas, between June 2016 and December 2017.
Methodology: Healthy and mature leaves were collected from *H. impetiginosus* in Alfenas/Minas Gerais, Brazil. Endophytic fungi were isolated from leaves by following standard microbiological methods. All isolated fungi (122) were used in the screening for potential lipase production. Submerged fermentation cultivations, using two different carbon sources, were carried out based on the previous screening results obtained. The best lipase-producing endophytic fungus was identified

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using molecular biology techniques. The produced lipase by submerged fermentation was purified by organic solvent precipitation and characterized by SDS-PAGE analysis.
Results: A total of 122 isolates of endophytic fungi were obtained. Two isolated fungi showed high lipase activity in the plate screening and were chosen for submerged fermentation cultivations using glucose and cottonseed oil as carbon sources. A maximum lipase activity of 5.9 U/mL was obtained for one strain after 48 h of fermentation for the culture medium using cottonseed oil as a carbon source. This strain was genetically identified as *Preussia africana*. A single protein band with an apparent molecular mass of 64 kDa was detected by SDS-PAGE analysis after lipase purification (purification factor of 18.5).
Conclusion: A potential microorganism, able to produce an extracellular lipase in submerged fermentation, was isolated from *H. impetiginosus*. To date, this is the first report of extracellular lipase production by *Preussia africana*. The potential for this new lipase should be evaluated through a full characterization of these lipase properties in further studies.

Keywords: Endophytic fungi; fermentation; lipase; Preussia sp.

1. INTRODUCTION

Endophytes are microorganisms that colonize different plant tissues, such as leaves, stems, bark, roots, fruits, flowers, and seeds, for at least part of their life cycles, without deleterious effects on their hosts [1]. Generally, they have a symbiotic relationship with the host; while the host plant benefits from a large amount of compounds that provide protection against herbivore or pathogen attacks, the endophytes receive shelter and nutrients [2]. Also, this colonization by endophytic fungi might contribute to the plant adaptation to abiotic stress factors such as heavy metal toxicity and salinity [3,4].

The tree species *H. impetiginosus* belongs to the family Bignoniaceae and is commonly known as red lapacho or taheebo. It is a medicinal plant native to the Amazon rain forest and widespread throughout Central and South America [5]. In traditional medicine, the inner bark of this plant has been used by rural communities of Brazil for centuries for the treatment of a large number of diseases [6]. The extensive use of *H. impetiginosus* has motivated several studies on its extracts; endophytes of this species are a potential source of secondary metabolites and enzymes with high value for the pharmaceutical industry [2,7,8].

However, despite the large number of studies, endophytic strains are still not adequately described, taking into account that Hawsworth and Rossman [9] estimated that more than one million species of endophytes may exist. Besides, a considerable amount of work has been carried out exploring the diversity of endophytic fungi, but only a few studies reported their potential as new sources of industrially useful enzymes such as lipases, phytases, proteases and amylases [2,10,11,12,13]. Marques et al. [14] reported the production of cellulases and xylanases by 14 strains of endophytic fungi by solid-state fermentation (SSF), using lignocellulosic materials as substrates. The authors concluded that enzymatic extracts of *Botryosphaeria* sp. (AM01) and *Saccharicola* sp. (EJC 04) have potential for application in pretreated sugarcane bagasse saccharification processes. So we can therefore safely say that plant-associated endophytic fungi are still an unexplored source of natural substances and enzymes.

The application of enzymes in industrial processes has considerably advanced during recent years. Characteristics such as high catalytic efficiency, specificity, and the ability to accelerate specific chemical reactions without the formation of undesirable by-products are aspects that have contributed to increase the use of these biocatalysts in different industrial sectors [15]. Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) have the natural function of catalyzing the hydrolysis of triglycerides into diglycerides, monoglycerides, glycerol, and free fatty acids. The promising application of lipases in industrial processes is due to their versatility in catalyzing reactions in aqueous (hydrolysis) and also inorganic (esterification, transesterification, and interesterification) media. Moreover, many lipases have shown high specificity, providing products that could not be obtained by conventional chemical processes [15,16].

Sunitha et al. [17] isolated from medicinal plants fifty endophytic fungal strains, which were screened for extracellular enzymes such as amylase, cellulase, laccase, lipase, pectinase

and protease on solid media. Fifty percent of fungi screened for enzymes showed positive for lipase. Toghueo et al. [18] also reported the isolation of 87 endophytic fungi from medicinal plants and their screening for enzymatic activity such as amylase, cellulase, lipase, and laccase. Lipase activity was detected in 59.8% of the isolates tested. The authors concluded that these microorganisms are potential enzymes-producing of industrial interest.

Therefore, the present study was carried out to isolate and identify endophytic fungi from *H. impetiginosus* and to evaluate their ability to produce extracellular lipases by submerged fermentation. To the best of our knowledge, this is the first report about endophytic fungi isolated from *H. impetiginosus*, furthermore, studies aiming to find new enzymes produced by endophytic fungi are still scarce.

2. MATERIAL AND METHODS

2.1 Plant Samples

Healthy and mature leaves were collected from *H. impetiginosus* (Mart. ex DC.) Mattos in Alfenas/Minas Gerais (S21°25'16.95", W45°57'05.15""), Brazil. The plant was identified by Dr. Lúcia G. Lohmann of the Botanical Department of the Bioscience Institute of the University of São Paulo. A voucher specimen was deposited at the Federal University of Alfenas herbarium under the identification number 2745. Leaves were collected, transported to the laboratory, and processed within a few hours after sampling.

2.2 Isolation of Endophytic Fungi

The leaves were washed under running tap water and dried at room temperature. In a sterile environment, in order to eliminate epiphytic microorganisms, they were dipped in 70% ethanol for 1 min, followed by 2.5% (v/v) sodium hypochlorite solution for 6 min and 70% ethanol for 1 min. Subsequently, the leaves were rinsed with two changes of sterile distilled water. To assess whether disinfection methods were effective, 100 µL of the last washing water were inoculated into potato dextrose plates (ABD) supplemented with 0.1 g/L chloramphenicol and incubated at 28°C; no fungal growth was observed [19].

After washing, the leaves were cut into small fragments of approximately 5 mm in length. The obtained fragments were transferred to potato dextrose agar (PDA) plates supplemented with 0.1 g/L chloramphenicol to inhibit bacterial growth; plates were incubated at 28°C. After five days, parts of the mycelium emerging from the leaf fragments were transferred to PDA tubes without chloramphenicol. Tubes were incubated at 28°C until the fungus spread over the entire agar surface and were then kept refrigerated at 4°C [19].

2.3 Endophytic Fungi Screening for Lipase Production

All 122 endophytic fungi isolated from leaves and stored at 4°C in PDA tubes were used in the screening for potential lipase production, using Tween 20 as substrate [20]. A suspension of spores in the saline solution was prepared from a fresh endophytic fungi culture previously isolated (optical density OD_{600} = 0.05). To estimate lipolytic activity, 10 microliters of the spore suspension were inoculated on Petri dishes with suitable medium, consisting of peptone 1% (m/v), NaCl 0.5% (m/v), $CaCl_2$ 0.1% (m/v), agar 1.5% (m/v), and Tween 20 1.0% (v/v), and incubated at 28°C for 120 h [20]. Assays were carried out in triplicate.

Lipase activity (Pz) was defined as the ratio between colony size and halo size formed around the colony; both parameters were measured after seven days. Lipase activity was interpreted as follows: Pz < 0.61 as "++" (highly positive activity);0.61 < Pz < 1 as "+" (middle positive enzyme activity); and Pz = 1 as "–" nondetected activity [21].

Finally, submerged fermentation cultivations were carried out based on the screening results using plating assays.

2.4 Identification of Endophytic Fungus

The Hi2.A8 strain was identified using molecular biology techniques. Genomic DNA was extracted according to the protocol described by Raeder and Broda [22] and used as template to amplify ITS data via PCR. The amplified fragments were purified and sequenced on ABI3500XL Series (Applied Byosystem). The primers ITS-1 and ITS-4 were employed for amplifying and sequencing, and the obtained sequences were assembled in contig and compared with microorganism sequences from Genbank (http://www.ncbi.nlm.nih.gov) and CBS (Centraalbureau voor Schimmelcultures, Fungal

Biodiversity Center) databases (http://www.cbs.knaw.nl/). The sequences were aligned by CLUSTAL X [23] and phylogenetic analyses were conducted using MEGA version 6.0 [24]. Evolutionary distance was estimated by the Kimura [25] model and phylogenetic reconstruction was performed using the Neighbor-Joining method [26], with bootstrap values calculated from 1,000 replicate runs, using MEGA 6.0.

2.5 Lipase Production by Submerged Fermentation

The strains Hi2.A8 and Hi1.J1 kept in PDA tubes were reactivated on Sabouraud Dextrose Agar (SDA) plates at 28°C for 96 h. A colony with a diameter of 5 mm was removed from the SDA plate and inoculated into a medium composed of 2% (m/v) peptone, 1.5% (v/v) olive oil, 0.1% (m/v) yeast extract, and 0.05% (m/v) each $MgSO_4$ and $NaNO_3$. Extracellular enzyme production was carried out for 72 h at 28°C and 250 rpm, with 10% (v/v) inoculum of the selected strains in 1000-mL shaker flasks with 90 mL sterilized culture medium. Samples were taken in duplicate at 12 h intervals and filtered under vacuum; the resulting supernatant was used for analysis of lipase activity, pH, and cell concentration. To determine cell concentration, the suspension was filtered (Whatman paper n° 44), followed by washing with distilled water. The washed cells were dried at 45°C until constant mass.

A basic medium containing peptone 2% (m/v), yeast extract 0.1% (m/v), $NaNO_4$ 0.05% (m/v), $MgSO_4$ 0.05% (m/v), and cottonseed oil as inducer 0.5% (v/v) was used. A different carbon source was used in each medium; glucose 0.5% (v/v) for the first assay and cottonseed oil 0.5% (v/v) for the second assay [27]. Therefore, cottonseed oil was used as both carbon source and inducer in the second medium. The use of raw materials with lower costs, such as cottonseed oil, makes lipase production more economic; raw materials usually account for 25–50% of the total lipase production costs.

2.6 Lipase Purification by Organic Solvent Precipitation

The lipase previously filtered was firstly concentrated by vacuum centrifugation (Labconco CentriVap Centrifugal Vacuum Concentrators & Cold Trap). Then cold acetone (0°C) was added into this concentrated supernatant (acetone:crude extract ratio of 2:1) in order to precipitate the lipase. This system was vortexed and centrifuged for 20 min at 1000 g for phase separation. The resulting precipitate was resuspended in 100 mM phosphate buffer at pH 7.0. The purification factor (F) was calculated as the ratio between the specific activity (IU/mg) for purified lipase and crude extract. Also, electrophoresis analysis was carried out to verify the enzyme purity. The recovery percentage (R) was calculated as the ratio of the total lipase activity (IU) between purified lipase and crude extract multiplies by 100.

2.7 Determination of Lipase Activity

Lipase activity was determined by hydrolysis of the olive oil emulsion [28]. The substrate was prepared by mixing 50 mL of olive oil with 50 mL of Arabic gum solution 7% (m/v). The reaction mixture containing 5 mL of the emulsion, 4 mL of 100 mM sodium phosphate buffer at pH 7.0, and 1 mL of supernatant (filtered after fermentation) was incubated for 5 min at 37°C under continuous agitation in an orbital shaker (200 rpm). The reaction was stopped by adding 15 mL of acetone and ethanol mixture 1:1 (v/v). The liberated fatty acids were titrated with standard 20 mM sodium hydroxide solution in the presence of phenolphthalein as indicator. Reaction blanks were prepared by adding 1 mL of culture medium. One international unit (IU) of activity was defined as the amount of enzyme required to liberate 1 µmol of fatty acids per minute under the experimental conditions described above.

2.8 SDS-PAGE Analysis

SDS-PAGE analysis was performed in a Mini-Protean II Dual-Slab Cell (BioRad, USA) according to Laemmli [29]. The analysis was performed by using 12% polyacrylamide for the stacking and resolving gels, respectively. Low range molecular mass standards (14,4 a 97,4 kDa) from BioRad were used. The gel was stained with Coomassie Brilliant Blue R-250.

3. RESULTS AND DISCUSSION

3.1 Isolation and Screening

Overall, 122 isolates of endophytic fungi were obtained from the leaf pieces of *H. impetiginosus*. All isolated fungi were subjected to lipase production screening. Thus, 122 isolated fungi were properly classified according

to the halo size around the colony or the absence of it (Table 1).

Based on our results, 52 isolated fungi did not show lipase activity (Pz = 1), 68 showed moderate lipase activity (0.61 < Pz < 1), and only two showed high lipase activity (Pz < 0.61). Strains Hi2.A8 (n° 8, Table 1) and Hi1.J1 (n° 120. Table 1), with high lipase activity were selected for the submerged fermentation assays.

3.2 Lipase Production by Submerged Fermentation

Submerged fermentation was carried out to verify the capacity of selected microorganisms for extracellular lipase production. This method presents advantages such as simple cultivation at large scales, homogeneity of the medium, and easy control of important parameters [30]. In general, the amount of lipase produced by the microorganisms depends on factors such as the culture medium composition, pH, temperature, oxygenation, and agitation of the fermentation medium. It is also common to use substances known as inducers, such as vegetable oils, fats, and fatty acids [15, 27]. Thus, two different carbon sources (glucose and cottonseed oil) were evaluated by submerged fermentation in order to verify extracellular lipase production ability.

The two selected fungus strains (Hi2.A8 and Hi1.J1) were able to grow in the fermentation media with different carbon sources (glucose and cottonseed oil; Figs. 1 and 2). This means that both endophytic fungi can potentially metabolize cottonseed oil as a source of carbon since in one of the media tested, only cottonseed oil was used as a carbon source. However, extracellular lipases were only detected for *Preussia africana* (Hi2.A8), with highest lipase activity (5.9 IU/mL)

Table 1. Screening of lipase production by endophytic fungi using solid media

nº	Fungi	LP	nº	Fungi	LP	nº	Fungi	LP	nº	Fungi	LP
1	Hi2.A1	-	32	Hi2.E2	+	63	Hi2.L7	-	94	Hi1.B1	+
2	Hi2.A2	+	33	Hi2.E4	+	64	Hi2.L8	+	95	Hi1.B2	-
3	Hi2.A3	-	34	Hi2.E5	+	65	Hi2.M1	+	96	Hi1.B3	+
4	Hi2.A4	+	35	Hi2.F1	-	66	Hi2.M2	+	97	Hi1.B4	+
5	Hi2.A5	-	36	Hi2.F2	+	67	Hi2.M4	+	98	Hi1.B5	+
6	Hi2.A6	-	37	Hi2.F3	+	68	Hi2.M5	-	99	Hi1.C1	+
7	Hi2.A7	-	38	Hi2.F4	+	69	Hi2.M6	+	100	Hi1.C2	-
8	Hi2.A8	++	39	Hi2.G1	-	70	Hi2.N1	+	101	Hi1.D1	-
9	Hi2.A9	+	40	Hi2.G4	-	71	Hi2.N2	-	102	Hi1.D3	-
10	Hi2.B1	+	41	Hi2.G6	-	72	Hi2.N3	-	103	Hi1.D4	-
11	Hi2.B2	+	42	Hi2.G7	-	73	Hi2.O1	+	104	Hi1.E3	+
12	Hi2.B3	+	43	Hi2.H1	+	74	Hi2.O2	-	105	Hi1.E4	+
13	Hi2.B4	+	44	Hi2.H2	+	75	Hi2.O3	-	106	Hi1.E5	+
14	Hi2.B5	-	45	Hi2.H4	-	76	Hi2.O4	-	107	Hi1.F1	+
15	Hi2.B6	+	46	Hi2.H5	-	77	Hi2.P1a	-	108	Hi1.F3	+
16	Hi2.B7	-	47	Hi2.H6	-	78	Hi2.P1	+	109	Hi1.F4	+
17	Hi2.B9	-	48	Hi2.I1	-	79	Hi2.P2	-	110	Hi1.F5	+
18	Hi2.C1a	-	49	Hi2.I2	-	80	Hi2.P3a	+	111	Hi1.G1	+
19	Hi2.C1	-	50	Hi2.I3	+	81	Hi2.P3	-	112	Hi1.G2	+
20	Hi2.C2	-	51	Hi2.I4	+	82	Hi2.R1a	-	113	Hi1.G3	-
21	Hi2.C3	-	52	Hi2.J1	-	83	Hi2.R1	+	114	Hi1.H1	+
22	Hi2.C4	+	53	Hi2.J3	+	84	Hi2.R3	-	115	Hi1.H4	+
23	Hi2.C5	+	54	Hi2.J4	-	85	Hi2.R4	+	116	Hi1.I1	+
24	Hi2.D1	+	55	Hi2.K4	+	86	Hi2.R5	+	117	Hi1.I2	+
25	Hi2.D2	+	56	Hi2.K5	-	87	H1.A1	+	118	Hi1.I3	+
26	Hi2.D3	-	57	Hi2.K6	+	88	Hi1.A3	-	119	Hi1.I4	-
27	Hi2.D4	+	58	Hi2.K7	+	89	Hi1.A4	+	120	Hi1.J1	++
28	Hi2.D5	-	59	Hi2.L2	-	90	Hi1.A5	+	121	Hi1.J3	-
29	Hi2.D6	+	60	Hi2.L3	+	91	Hi1.A6	+	122	Hi1.J4	+
30	Hi2.D7	+	61	Hi2.L5	-	92	Hi1.A7	-			
31	Hi2.E1	+	62	Hi2.L6	+	93	Hi1.A8	+			

"–" nondetected, "+"middle, "++" high; LP = lipase production

being obtained after 48 h of fermentation in the assay that contained the only cottonseed as carbon source.

Generally, extracellular lipase production is associated with cell growth. When only cottonseed oil was used as both carbon source and inducer (culture medium I) the lipase production was observed at the beginning of the fermentation process (Fig. 1a). However, when glucose was added as a carbon source (culture medium 2 – Fig. 1b), the lipase production was only observed after 72 h of fermentation (1.4 IU/mL; Fig. 1b). According to Li and Zong [31], lipase production can be limited by catabolic repression when glucose is used as a carbon source, which explains the low lipase activity found in this assay.

These results showed that cottonseed oil is an adequate alternative carbon source; it is cheaper than olive and soy oils and also it is able to induce lipase production with no catabolic repression.

For *Penicillium* sp. (Hi1.J1), none extracellular lipase activity was detected throughout the fermentation period for both culture media used (Fig. 2a and b). However, cellular growth was observed in these two assays. In the first case, we might assume that the glucose was responsible for microorganism growth, but in the second assay, when only cottonseed oil was used as carbon source, the microorganism must have hydrolyzed the oil and metabolized it as carbon source for its growth. This fact suggests that this evaluated strain has produced a mycelium-bound lipase, which explains its cellular growth with the absence of extracellular lipase in the fermentative broth. Endophytic fungi isolated from Mediterranean plants by Torres et al. [32] have been described as a source of mycelium-bound lipases.

The pH values for both fungi strains increased throughout the fermentation period, probably due to the degradation of nitrogen sources present in both culture media. The aim of the present study was to search for lipase-producing endophytic fungi isolated from *H. impetiginosus*; in this context, only the Hi2.A8 strain was genetically identified. Hi1.J1 strain was identified as *Penicillium* sp. according to macroscopic (morphology, size, coloration of the mycelium) and microscopic (presence of spores or other reproductive structures) characteristics. We plan to focus on mycelium-bound lipase producers and on the optimization of the culture media in a further study.

3.3 Identification of Endophytic Fungus

The partial sequence of the ITS1-5.8S-ITS2 region of Hi2.A8 showed 99-100% similarity to the sequences from the same operon ribosomal region of the *Sporormiaceae* (Pleosporales – Ascomycota) contemplating some species of *Preussia*, deposited in the Genbank and CBS databases (Fig. 3). This partial sequence was deposited in Genbank (Genbank Biosample accession: SAMN 06350896).

Phylogenetic analysis (Fig. 4) recovered Hi2.A8 in a grouping, consistent with the *Preussia africana* sI2 line (AY 510420) reported by Arenal [33]. However, as the result of the analysis

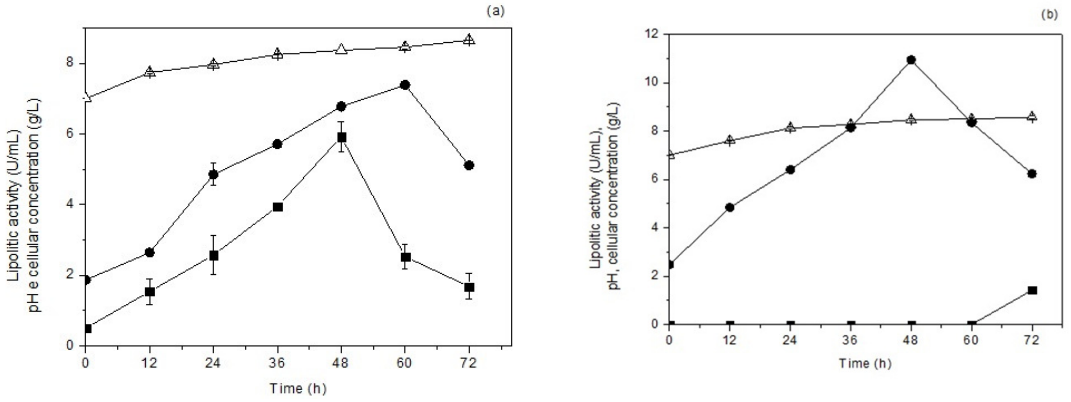

Fig. 1. Time courses of cellular concentration (●), pH (Δ), lipase activity (■) obtained for lipase production from *Preussia africana* (Hi2.A8) during 72 h of submerged fermentation using: a) cottonseed oil as carbon source; b) glucose as carbon source

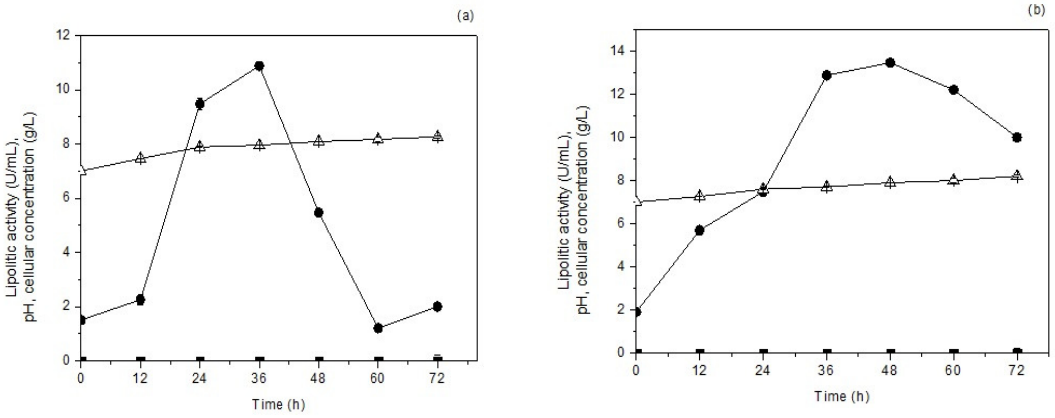

Fig. 2. Time courses of cellular concentration (●), pH (Δ), lipase activity ( ) obtained for lipase production from *Penicillium* sp. (Hi1.J1) during 72 h of submerged fermentation using: a) cottonseed oil as carbon source; b) glucose as carbon source

CCCTTGCCTTTTTGAGTACCTTTTCGTTTCCTCGGCAGGCTCGCCTGCCAATGGGG
ACCCCAACAAACACTTTGCAGTACCTGTAAACAGTCTGAACAAACTTTTAAAAATT
AAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAA
TGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCTTTGGTATTCCTTAGGGCATGCCTGTTCGAGCGTCATTGAAACCTTC
AAGCTCAGCTTGGTGTTGGGTGACTGTCCGCTTGCGGACTCGCCTCAAAATGATT
GGCGGCCGGTACTTTTGGCTTCGAGCGCAGCAGAAACGCGAACTCGAGGCCTGT
GTGCTGGCTCCCAGAAGCTATCTTCACAATTTTGACCTCGGATCAGGTAGGGATA
CCCGCTGAACTTAAGCATATCAATAAG

Fig. 3. Ribosomal operon partial sequence of Hi2.A8 strain (CPQBA 1304/16 DRM – 01)

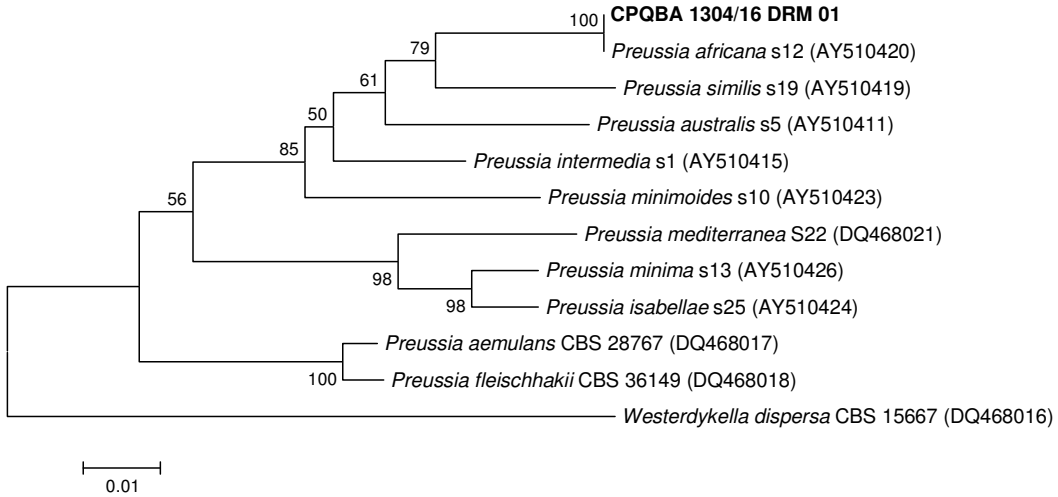

Fig. 4. Neighbor-Joining (NJ) phylogenetic tree showing phylogenetic relationships between ITS1-5.8S-ITS2 ribosomal operon partial sequence of Hi2.A8 strain (CPQBA 1304/16 DRM – 01) and related microorganisms available in GenBank and CBS database

performed with data from the databases does not corroborate with the phylogeny, the sample should be identified as *Preussia* c.f. (to confirm) *africana.*

The genus *Preussia* is a poorly understood taxon, albeit being environmentally diverse [34]. *Preussia africana* was first isolated by Arenal [35] from plant debris and coprophilous substrata. However, two years later, Arenal [33] described the isolation of a new endophytic fungus from Mediterranean plants, named *Preussia mediterranea*. The authors affirmed that *Preussia mediterranea* was particularly similar to *Preussia africana* and two other species of *Preussia*.

Brum et al. [36] reported the isolation of *Preussia africana*, as an endophytic fungus, from *Vitis labrusca L.* 'Niagara Rosada' in São Paulo, Brazil. Similarly, Zaferanloo et al. [37] reported a variety of endophytes, isolated from Australian native plants, as a possible source of industrially useful enzymes. The authors conclude that in particular, the endophyte *Preussia minima* (EL-14) produced functionally diverse enzymes with respect to temperature and pH tolerance. In general, industrial processes require robust enzymes able to act within a wide range of conditions, such as extreme pH and temperature values [10]. In fact, different endophytic fungi potentially produce amylase, cellulase, lipase, and laccase [18].

3.4 Lipase Purification by Organic Solvent Precipitation

After the concentration of filtered fermentative broth (4 fold mL/mL) the lipase was precipitate with cold acetone (0℃) The recovery percentage obtained was 77.15% and the purification factor was 18.5. The electrophoresis analysis confirmed the purification of this lipase from fermentative broth once one single band appeared in the gel (Fig.5).

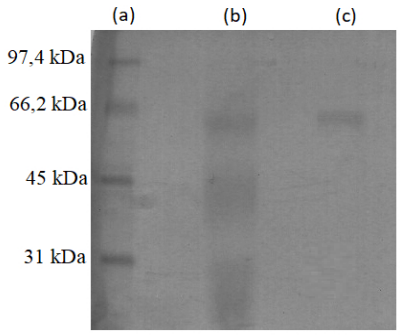

Fig. 5. SDS–PAGE analysis – Line: (a) Molecular mass markers; (b): Fermentation broth after concentration; (c) Purified lipase after precipitation step

4. CONCLUSION

It was possible to isolate, from *H. impetiginosus*, a potential microorganism able to produce an extracellular lipase in submerged fermentation. A maximum lipase activity of 5.9 U/mL was obtained after 48 h of fermentation for the culture medium using cottonseed oil as carbon source. This strain was genetically identified as *Preussia africana*. A single protein band with an apparent molecular mass of 64 kDa was detected by SDS-PAGE analysis after lipase precipitation by acetone (purification factor of 18.5). To date, this is the first report of extracellular lipase production by *Preussia africana*. The potential for this new lipase should be evaluated through a full characterization of these lipase properties in further studies.

ETHICAL APPROVAL AND CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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