

Fungos amazônicos produtores de celulases: uma revisão

Denilso Ibiapino Fontes¹, Darlisson de Alexandria Santos²

Resumo

Os fungos são a principal fonte natural de celulases, sendo eles o objeto de estudos que visam a descoberta de linhagens mais eficientes na produção destas enzimas. No entanto, embora o ambiente amazônico seja uma das maiores fontes de biodiversidade do planeta, os fungos oriundos deste ambiente ainda não tiveram seu potencial biotecnológico verdadeiramente avaliados, sobretudo no que diz respeito a produção de enzimas de interesse comercial. Desta forma, neste artigo apresentamos uma revisão da literatura acerca dos fungos isolados do ambiente amazônico que foram relatados como produtores de celulase, sendo observado que são poucos os estudos de prospecção com fungos deste bioma e consequentemente um número reduzido de espécies foram relatadas como produtoras de celulases. Mesmo com poucos estudos, ficou claro que há um enorme potencial biotecnológico ainda inexplorado, pois algumas das linhagens mostraram capacidade de produção de celulases semelhantes aos microrganismos já utilizados comercialmente.

Palavras-chave: Celulases, Floresta Amazônica, Biotecnologia

Amazonian fungi as cellulase producers: a review. Fungi are one of the main natural sources of cellulases, and the discovery of new funai strains with more effective application in cellulase production is the main goal of many scientific studies. Since the Amazon environment is one of the greatest resources of biodiversity on Earth, fungi from this environment might present great potential for cellulase production. However, their biotechnological potential has not been truly studied, especially as related to the production of enzymes with high commercial interest. Therefore, in the present paper, we present a literature review of all the fungi strains from the Amazon environment that have already been reported in the literature as cellulase producers. It is possible to note that only a few prospective studies have been carried out, and as consequence, few fungi strains were related to cellulase producers. Despite the scarce number of strains reported with such potential, some of the strains studied showed excellent results, like those being applied commercially. These observations led to the conclusion that a huge biotechnological potential is hidden inside the Amazon forest and if new prospective studies were carried out, new potentials would be discovered.

Keywords: Cellulases, Amazon Forest, Biotechnology

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1. Introduction

Since the 1990s, cellulases have been responsible for more than 20% of the world enzyme market. They are used as a food additive for animal feed, increasing the digestibility of ruminants (cattle, goats, etc.) and in the pulp and paper industry, the textile sector (during many production steps), detergents (mainly in those applied to treat cotton fibers), and in beverage production, promoting the clarification of fruit juices and wines (DEKA et al., 2011; KUHAD et al., 2011). However, many current studies are aimed at the use of in second-generation cellulases production ethanol through the hydrolysis of lianocellulosic biomass.

These studies are supported by the growing demand for renewable fuels, especially bioethanol. This is supported by US Department of Energy projection in 2012 that predicted 30% of petroleum-based fuels for combustion engines will be replaced by fuels derived from biomass by the year of 2025 (LIMAYEM & RICKE, 2012). Even if this projection does not materialize, the demand for biofuels will certainly be greater than the current demand. This can lead to an increase in the cultivated area, whether with corn or sugarcane which are the two main raw materials for the alobal production of ethanol (LIMA et al., 2015).

To increase bioethanol production without increasing the planted area, the prospect of cellulaseproducing microorganisms is one of the possible strategies to obtain better enzymes for the hydrolysis of lianocellulosic materials. This would contribute to the feasibility of cellulosic ethanol production, which is one of the most promising technologies under development (BASTOS, 2007).

Cellulose is the most abundant polymer in the biosphere and the main constituent of lignocellulosic biomass, corresponding to one-third to one-half of plant tissue (SUN & CHENG, 2002; SÁNCHEZ & CARDONA, 2008). In addition, cellulose has gained greater attention, mainly due to its homogeneity. Since it is composed only of glucose units, it is more susceptible to the development of methodologies for its use in bioethanol production on an industrial scale (LYND et al., 2002).

To carry out enzymatic cellulose hydrolysis, the cellulases act synergistically because they are comprised of an enzyme complex with different roles for each type of enzyme: endo-1,4-β-D-glucanases internally hydrolyze the cellulosic chain in a random way decreasing its degree of polymerization; exo-1,4-β-D-glucanases hydrolyze the cellulosic chain from its ends, releasing cellobioses; and 1,4-β-D-glucosidases promote the hydrolysis of cellobiose into alucose, and they perform cleavage also of can glycosidic units from cellooligosaccharides (OLSSON & HAHN-HÄGERDAL, 1996; OGEDA & PETRI, 2010).

The aim of this work was to review all of the articles and other reports of Amazonian fungi strains as cellulase producers that have been published and are available on the main search platforms.

2. Methods

For this review, a search was performed on the following platforms: Portal Periódicos, Google Scholar, ScienceDirect, Springer, Wiley Online Library, and SciELO. The search involved all of the articles and other reports whose studies were aimed at prospecting for Amazonian



microorganisms capable of producing cellulases, with no publication year restriction. The studies with a few strains or a single strain were also included in this search.

The keywords for the search in the different platforms were as follows: "Amazonian fungi cellulases", "Amazon cellulases", "fungos amazônicos produtores de celulases" e "cellulases de fungo amazônico". All of the articles from scientific journals, book chapters, and theses published in English or Portuguese languages were included in this work. Congress communications were not considered in the present report, and patents were not found in previous searches.

3. Fungi as cellulase producers

There is a wide range of microorganisms capable of producing cellulases. They have been reported by many studies in the literature. Although bacteria are the main producers reported, the Bacteria Genome Database (CAZy) showed that 38% of them contain at least one enzyme of the cellulase complex, filamentous fungi have also been studied and reported as great cellulase producers (JUTURU & WU, 2014).

Filamentous funai are also applied other biotechnological in many because of their processes physiological, enzymological, and biochemical properties that allow them to grow on solid substrates. These substrates are bio-converted into a large number of products, and they are also responsible for increasing the hydrolysis capacity of microorganisms because they are a great source of nutrients (SOCCOL et al., 1994).

The main fungal genera described in the literature (Table 1) as cellulase producers are *Trichoderma*, *Aspergillus*, *Penicillium*, and *Fusarium*. These are mostly cultivated in solidstate culture medium because this type of culture medium favors a greater production of this enzyme complex (SUKUMARAN et al., 2009; DELABONA et al., 2012a; DHILLON et al., 2012; SAJITH et al., 2014; BELMONT-MONTEFUSCO et al., 2020).

 Table 1. Example of fungi genera reported in the literature as cellulase producers.

Strain	Reference	
Aspergillus terreus UniMAP AA-6	Gunny et al. (2015)	
Aspergillus flavus BS1	Sajith et al. (2014)	
Acremonium cellulolyticus	FAO (1997)	
Trichoderma asperellum RCK2011	Raghuwanshi et al. (2014)	
Aspergillus oryzae NRRL 3484	El-Ghonemy et al. (2014)	
Aspergillus fumigatus	FAO (1997)	
Melanoporia sp. CCT 7736	De Oliveira et al. (2016)	
Trichoderma harzianum P49P11	Delabona et al. (2012a)	
Trichoderma viride QM 9414	Lan et al. (2013)	

The filamentous fungus Trichoderma reesei RUT C30 has already been reported to have the greatest potential for the production of cellulases. However, an industrial-scale cellulose hydrolysis system with this strain, that would be economically viable if compared to current bioethanol production technologies, has not yet been developed (MEKALA



et al., 2008; SUKUMARAN et al., 2009). strain usually produces two This cellobiohydrolases (CHB I and CHB II) and two endoglucanases (EG1 and EG2) in an approximate ratio of 6:2:1:1. Together, these add up to 90% of the cellulase cocktail. However, all of the βglycosidases secreted by this fungus reach less than 1%, requiring the use of genetic engineering techniques to increase their production (MARGEOT et al., 2009). In addition to the low β glycosidases production, the T. reesei RUT C30 strain is inhibited by the sideproducts generated during the cellulose hydrolysis process (DELABONA et al., 2012a).

Even though numerous studies have been reported in the literature, there is no report of a fungal strain that economically competitive is for application in bioethanol production. Therefore, it is necessary to continue prospecting for new fungal strains capable of producing cellulases so that a viable strain can be found and make this process more advantageous. Thus, the discovery of new strains of microorganisms isolated from environments that are rich in decomposing biomass might be the answer to this problem. The Amazon forest is a great example of such an environment.

4. Amazonian fungi

The Amazon biome occupies almost half of the Brazilian territory and is considered the largest place of biodiversity on the planet, corresponding to about 20% of all existing biodiversity, whether plants, animals, or microorganisms (THIEME et al., 2007). The Amazon Forest soil is very rich in decomposing organic biomass due to the great amount of leaves and other parts of trees that regularly fall on the soil. Also, the high humidity of the region allows the decomposing biomass to be a substrate with great potential for the growth of a great diversity of fungi strains (ALVIRA et al., Despite 2010). the great fungal biodiversity in the Amazon environment, the number of studies aimed at the prospection and selection of fungi strains with biotechnological potential is very small, and these few studies point to the existence of strains with great potential in the production of cellulases, as we will see below.

One of the first prospecting studies of Amazonian fungi was described by CARVALHO et al. (1992). In this study, the fungi strains were isolated from decaying wood piles in the city of They Manaus-AM, Brazil. were identified, and their production of lignocellulolytic enzymes was evaluated, with greater attention to Tissue cellulases. filter paper, carboxymethylcellulose, and pnitrophenyl-b-D-alycopyranoside were used as substrates to respectively measure FPAse, endo-glucanase, and b-glycosidase activities). Among the eight species reported, seven produced one or more type of (Table 2). The cellulases species Trichoderma pseudokoningii and Gliocladium virens were the only two strains that presented all cellulase types at the same time, and the former was applied in further studies (CARVALHO et al., 1992; FURLANETO & PIZZIRANI-KLEINER, 1992; NADALINI et al., 1999).

A second prospecting study has also brought to light fungi strains with great potential for producing cellulase. It was performed by DELABONA et al. (2012b). This study describes the isolation, screening, and selection of fungi species capable of degrading Amazon Forest biomass. In addition, they analyzed the cellulolytic enzyme



complex produced by the Aspergillus fumigatus P40M2 strain cultivated using different agro-industrial residues (wheat bran, sugarcane bagasse, soybean bran, and orange peel) as solid-state fermentation substrates (SSF). The activities achieved were 160.1 IU g⁻¹ for CMCase, 5.0 FPU g⁻¹ for FPase, and 105.82 IU a^{-1} for β -alucosidase. The characterization studies of the enzyme extract obtained showed that CMCase activity was higher at 65 °C and pH 3.0-3.5, indicating that A. fumigatus P40M2 produces a thermophilic and acidic endoglucanase.

The same research group mentioned above reported more fungi strains with great potential, such as Trichoderma harzianum P49P11, Aspergillus niger P47C3, Aspergillus oryzae P6B2, A. oryzae P27C3, and A. oryzae P40B3 (DELABONA et al., 2012a; DELABONA et al., 2012b; PIROTA et al., 2013a; DELABONA et al., 2013c; PIROTA et al., 2013b; PIROTA et al., 2014a). Among these strains, T. harzianum P49P11 has been the most studied due to its large production of cellulases, with similar potential to the T. reesei RUT C30 strain. In many studies, it is considered to the best be strain for cellulase production. In further studies, the T. harzianum P49P11 strain had its performance in cellulase producion improved by genetic engineering (DELABONA et al., 2012a; DELABONA et al., 2017; DELABONA et al., 2020).

Another Τ. harzianum strain isolated from the Amazon Forest was reported by SOUZA et al. (2018a). In this study, T. harzianum 422 was applied to good cellulase produce activity against sugarcane bagasse that was previously treated hydrothermally and with a ball mill. Therefore, this fungal species, even different strains, has good potential for use in cellulase production and consequently in bioethanol

production from lignocellulosic biomass.

Other prospecting studies were carried out by HARGREAVES (2008) (15 cellulase producing strains), SOUZA et (2008) (10 cellulase producing al. strains), LIMA et al. (2015) (12 cellulase producing strains), ARAÚJO et al. (2019) (16 cellulase producing strains), and TEIXEIRA et al. (2021) (42 cellulase producing strains). The prospecting reported by ARAÚJO et al. (2019) can be not only assigned to Amazon environment fungi but also to and endophytic epiphytic fungi, because the strains were isolated from cocoa cultivated in the state of Amazonas. Furthermore, the prospecting study reported by TEIXEIRA et al. (2021) involved fungi isolated from residues. pineapple crown This demonstrated that the potential of Amazonian fungi is not restricted to those isolated from soil or decaying organic matter but also from Amazon plants and fruits. In addition, the need for further studies of Amazonian fungi is almost mandatory because there is such great biodiversity and a large territorial expanse to explore.

The Amazon territorial expanse is not restricted to Brazil because it extends into to neighboring countries in South America. One example is the study carried out by VEGA et al. (2012) that evaluated fungi strains isolated from the soil of the Amazon rainforest in Peru for their potential as cellulase producers. Fifty different morphological colonies were selected by using the compensation plate assay with CMC as substrate, and eleven of these also showed cellulase production in liquid media at alkaline pH values. At the end, Aspergillus sp. LM-HP32, Penicillium sp. LM-HP33, and Penicillium sp. LM-HP37 were selected as the best cellulase



producers, with high specific yields (>30 g $L^{-1} h^{-1}$).

Other studies that were performed with only one Amazonian environment strain have also showed the great potential of this biome. For example, the study reported by PIROTA et al. (2016) evaluated the effects of solid-state fermentation (SSF) conditions in cellulase production by a new strain of Aspergillus oryzae P27C3. Their work was performed in a laboratory-scale bioreactor equipped with an automated system in line. It achieved the best production from FPase (0.40 IU g-1), endoglucanase (IU 123.64 g-1), and β -glucosidase (18.32 IU g-1) at 28 °C using a 70% moisture substrate.

PIMENTEL et al. (2014) studied a Penicillium citrinum strain that was

isolated from agro-industrial residues in the Amazon region, Presidente Figueiredo-AM. They observed its potential as a producer of cellulolytic (endoglucanases enzymes and exoglucanases). Using the same strain, SOUZA et al. (2014) purified and characterized an endoglucanase and showed the possibility of using this Penicillium citrinum strain to produce a commercial cellulase.

As evidenced so far, the potential of fungi from the Amazon environment in cellulase production is enormous, but it has been under-reported due to the low number of studies. A large part of the existing studies can be seen in Table 2.

Table 2. Prospecting studies of cellulase	production by	Amazonian funai
Table 2. Frospecting studies of Cellulase	production b	y Amazonian iongi.

Strain	Source (matrix and place)	Enzyme	Reference
Cladosporium cladosporioides	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Talaromyces macrosporus	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Fomitopsis subtropica	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Aspergillus versicolor	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Penicilliun rubidurum	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Talaromyces radicus	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Daldinia eschscholtzii	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Diaporthe pseudomangiferae	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Penicillium pimiteouiense	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Diaporthe phaseolorum	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Neonothopanus nambi	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Diaporthe tectonae	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Aspergillus parasiticus	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Marasmius cladophyllus	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Diaporthe lithocarpus	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Phyllosticta capitalensis	Cocoa seed, Pará state	CMCase	Araújo et al. (2019)
Trichoderma sp. T676	Amazon Forest soil	FPAse	Buzato et al. (2012)
Candida amazonensis sp.	Rotten wood, Roraima	-	Cadete et al. (2011)
Trichoderma Pseudokoningii	Decaying wood, Amazonas	FPAse, b- glicosidase, endo-glucanase	Carvalho et al. (1992 Furlaneto & Pizzirani- Kleiner (1992) Nadalini et al. (1999) Silva et al. (1999)
Gliocladium virens	Decaying wood, Amazonas	FPAse, b- glicosidase, endo-glucanase	Carvalho et al. (1992
Mucor sp.	Decaying wood, Amazonas	FPAse, b- glicosidase	Carvalho et al. (1992



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Ciências Biológicas

Fusarium axysporum Decaying wood, Amazonas endo-glucanase FPAse, b- glicosidase endo-glucanase Carvalho et al. [1992] Aspergillus flavus Decaying wood, Amazonas FPAse, b- glicosidase Carvalho et al. [1992] Thermoascus aurantiacus Decaying hemicellulosic material, Amazonas b-glicosidase Carvalho et al. [2010] Carvalho et al. (2014) Decaying hemicellulosic material, Amazonas b-glicosidase Carvalho et al. [2012) Trichoderma harzianum P49P11 Soil and decomposed wood samples, Pará FPAse, b- glicosidase Carvalho et al. [2012) Gelain et al. (2013), Gelain et al. (2013), Gelain et al. (2012), Gelain et al. (2013), Soil and decomposed wood samples, Pará FPAse, b- glicosidase Delabona (2013), Firota et al. (2013), Gelain et al. (2013), Gelain et al. (2014), Firota et al. (2014), Gelain et al. (2014), Firota et al. (2014), Firo	Trichoderma harzianum	Decaying wood, Amazonas	FPAse	Carvalho et al. (1992)
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Stereum sp. 4D2 Basidiomycetes from Campina CMC ase Sousa et al. (2008)	Pycnoporus sanguineus 12B	Basidiomycetes from Campina	CMCase	Sousa et al. (2008)
	Stereum sp. 6D2	Basidiomycetes from Campina	CMCase	Sousa et al. (2008)



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Stereaceae 22B	Basidiomycetes from Campina Reservation- INPA, Amazonas	CMCase	Sousa et al. (2008)
Daedalea sp. 1A	Basidiomycetes from Campina Reservation- INPA, Amazonas	CMCase	Sousa et al. (2008)
Pleurotus sp. 1C4	Basidiomycetes from Campina Reservation- INPA, Amazonas	CMCase	Sousa et al. (2008)
Daedalea sp. 4E6	Basidiomycetes from Campina Reservation- INPA, Amazonas	CMCase	Sousa et al. (2008)
Cantharellus guyanensis 4BI	Basidiomycetes from Campina Reservation- INPA, Amazonas	CMCase	Sousa et al. (2008)
Ganoderma applanatum 15B2	Basidiomycetes from Campina Reservation- INPA, Amazonas	CMCase	Sousa et al. (2008)
Trichoderma asperellum SB -2	Pineapple crown residue, Tocantins	FPAse, CMCase, b-glicosidase	Teixeira et al. (2021)
Trichoderma asperellum SB -11	Pineapple crown residue, Tocantins	FPAse, CMCase, b-glicosidase	Teixeira et al. (2021)
Frichoderma asperellum CMC-7	Pineapple crown residue, Tocantins	FPAse, CMCase, b-glicosidase	Teixeira et al. (2021)
Frichoderma asperellum PEC-2	Pineapple crown residue, Tocantins	FPAse, CMCase, b-glicosidase	Teixeira et al. (2021)
Trichoderma asperellum PEC-6	Pineapple crown residue, Tocantins	FPAse, CMCase, b-glicosidase	Teixeira et al. (2021)
Frichoderma asperellum PEC-17	Pineapple crown residue, Tocantins	FPAse, CMCase, b-glicosidase	Teixeira et al. (2021)
Aspergillus niger	Pineapple crown residue, Tocantins	FPAse, CMCase, b-glicosidase	Teixeira et al. (2021)
Pleorotus ostreatus	Pineapple crown residue, Tocantins	FPAse, CMCase, b-glicosidase	Teixeira et al. (2021)
Penicillium sp. LM-HP06	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Penicillium sp. LM-HP14	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Penicillium sp. LM-HP19	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Penicillium sp. LM-HP21	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Aspergillus sp. LM-HP29	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Aspergillus sp. LM-HP32	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Penicillium sp. LM-HP33	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Aspergillus sp. LM-HP34	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Penicillium sp. LM-HP37	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Penicillium sp. LM-HP43	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Aspergillus sp. LM-HP44	Macuya Forest, Pucallpa-Peru	CMCase, FPAse	Vega et al. (2012)
Colletotrichum graminícola	Amazon Forest soil	b-glicosidase	Zimbardi et al. (2013)
Penicilium citrionigrum	Coleção de Fungos da Amazônia – CFAM, FIOCRUZ Amazônia	CMCase	Lima et al. (2015)
Aspergillus niger	Coleção de Fungos da Amazônia – CFAM, FIOCRUZ Amazônia	CMCase	Lima et al. (2015)
Penicilium rugullosum	Coleção de Fungos da Amazônia – CFAM, FIOCRUZ Amazônia	CMCase	Lima et al. (2015)
Penicilium sp.	Coleção de Fungos da Amazônia – CFAM, FIOCRUZ Amazônia	CMCase	Lima et al. (2015)
	Soil from Tapajós Forest, Pará	FPAse, CMCase,	Hargreaves (2008)



Looking into the studies presented in Table 2, it is possible to observe that only 44 studies were aimed at evaluating cellulase production by Amazonian environment fungi, resulting in 76 strains being assigned with such potential. Despite this small number, it was possible to note that even a pathogenic fungus strain isolated from the Amazon environment can be a producer. cellulase good The Colletotrichum graminicola, whose genus is known as phytopathogen, was isolated from Amazon soil, and it produced good b-glucosidase activity using different lignocellulosic substrates (wheat bran, sugarcane bagasse, peanut husks, rice straw and corn straw) (ZIMBARDI et al., 2013).

Finally, the study reported by TOYAMA et al. (2018) describes the discovery of a □-glucosidase through a metagenomic analysis of the water from Poraquê Lake. This discovery shows that there is still a lot to be learned about the Amazonian microbiota and its biotechnological potential as cellulase producers.

5. Conclusions

In this review, it was possible to note known about the that little is biotechnological potential of fungi strains from the Amazon environment, especially their potential in regard to the production of cellulase, with only 44 studies reported in the literature. However, among these few studies, it is evidenced that there is great potential because some fungi strains, such as T. harzianum P49P11 and T. harzianum 422, have been found to have potential high those shown as as by microorganisms that are already being used to produce cellulase on a commercial scale, and these are considered to be the most promising

strains for application in bio-ethanol production. New studies, whether for prospecting or even for the improvement of the already known strains, can also help to preserve the Amazonian microbiota and its sustainable exploration, which is so important for the whole World.

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Divulgação

Este artigo é inédito e não está sendo considerado para qualquer outra publicação. O(s) autor(es) e revisores não relataram qualquer conflito de interesse durante a sua avaliação. Logo, a revista *Scientia Amazonia* detém os direitos autorais, tem a aprovação e a permissão dos autores para divulgação, deste artigo, por meio eletrônico.

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