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Biotechnology and its Applications for *Penicillium echinulatum*: a Systematic Review

Biotecnologia e Suas Aplicações em *Penicillium echinulatum*: uma Revisão Sistemática

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Abstract

In the course of 40 years of research, different strains of the filamentous fungus Penicillium echinulatum were employed in biotechnological studies at University of Caxias do Sul (UCS). This systematic literature review proposes a historic analysis of research findings on this fungus. Bearing this in mind, a methodology composed of four steps was adopted, including: (i) question formulation; (ii) repository election; (iii) paper filtration; and (iv) reading, organization, analysis and result interpretation. A total of 37 scientific articles and 12 theses and dissertations were referred. Based on the encountered papers, it was evidenced that most works citing P. echinulatum were published by a single research group at UCS. These studies focus mainly on lignocellulolytic enzymes, including enhancement of biotechnological processes and selection of hyper-productive lineages. Furthermore, P. echinulatum has also appeared in a variety of distinct studies. While the strains used in investigations by UCS are all derived from the 2HH wild isolate, other research groups report isolation of the same species from several different sources, including red algae, blue grape mold, ham, soil samples, cheese, and subglacial ice. Additionally, it was evidenced that integrative approaches are essential in modern taxonomy. In conclusion, this review allowed the construction of a global picture of P. echinulatum throughout science, emphasizing the promising biotechnological application in enzyme production.

Keywords: *Penicillium echinulatum. Lignocellulolytic Enzymes. Biotechnology. Fungi*

Resumo

No decorrer de 40 anos de pesquisa, diferentes linhagens do fungo filamentoso Penicillium echinulatum foram empregadas em estudos na área da biotecnologia na Universidade de Caxias do Sul (UCS). Esta revisão sistemática da literatura propõe uma análise histórica das investigações científicas com esse fungo. Desse modo, uma metodologia composta de quatro etapas foi adotada, incluindo: (i) formulação de questões; (ii) seleção de repositórios; (iii) escolha de artigos; e (iv) leitura, organização, análise e interpretação de resultados. Um total de 37 artigos científicos e 12 teses e dissertações foram encontrados. Baseado nos artigos referidos foi evidenciado que grande parte dos trabalhos citando P. echinulatum foram publicados por um único grupo de pesquisa na UCS. O grande foco desses estudos são enzimas lignocelulolíticas, incluindo o aprimoramento de processos biotecnológicos e seleção de linhagens hiperprodutoras. Além disso, a espécie P. echinulatum também foi utilizada em outros estudos. Enquanto as linhagens utilizadas na UCS foram todas derivadas do isolado selvagem 2HH, nos outros grupos de pesquisa essa foi isolada em diferentes locais, incluindo algas vermelhas, videira, presunto, amostras de solo, queijo, e gelo subglacial. Adicionalmente, é salientado que uma abordagem taxonômica integrativa é essencial para a correta identificação dos isolados. Em conclusão, esta revisão sistemática permitiu a construção de um panorama global do P. echinulatum na ciência, dando ênfase na aplicação biotecnológica promissora de produção enzimática.

Palavras-chave: *Penicillium echinulatum. Enzimas Lignocelulolíticas. Biotecnologia. Fungos.*

1 Introduction

Lignocellulolytic enzymes are catalysts that promote plant biomass degradation (CASTRO; PEREIRA JUNIOR, 2010; OGEDA; PETRI, 2010). This characteristic allows a wide range of biotechnology applications throughout a large diversity of industrial fields, including: textile industry (AUSTAD, 2018), agricultural input industry (JAYASEKARA; RATNAYAKE, 2019), beverage industry (GUPTA; RODRIGUEZ-COUTO, 2018), animal feed industry (OJHA; SINGH; SHRIVASTAVA, 2019), and pharmaceutical industry (SAMPATHKUMAR *et al*., 2019). Additionally, lignocellulases comprise essential components of second-generation bioethanol production from alternative biomass sources, such as, corn, wheat and rice straw, cotton seed hair, sugarcane and sorghum bagasse (WANG *et al*., 2012; GUPTA; VERMA, 2015; SALEHI; TAHERZADEH, 2015). Since filamentous fungi are able to produce an exceptional amount of specific enzymes, their importance in biotechnology applications is on the rise (BHATTACHARYA; BHATTACHARYA; PLETSCHKE, 2015; PUNT *et al*., 2002).

The 2HH wild-type strain of *Penicillium echinulatum*, a filamentous fungus, was isolated in 1979 from the digestive tract of Coleoptera larvae *Anobium punctatum*, in the city of Caxias do Sul, Brazil (CARRAU et *al*., 1981). This event led to 40 years of multiple research endeavours with a biotechnology emphasis, having the enhancement of lignocellulolytic enzyme

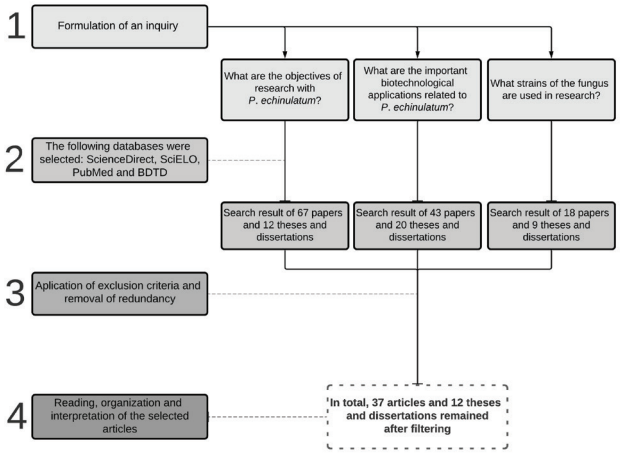
secretion as a main objective. Considering this, an emphasis was given on the improvement of strains and bioprocesses. Therefore, numerous mutant strains were created, from which two stand out: *(i)* The 9A02S1 strain, obtained through exposure of the wild-type strain to ultraviolet light, methyl methane thiosulfate and hydrogen peroxide (DILLON *et al*., 2006); *(ii)* and the S1M29 strain, acquired from hydrogen peroxide mutagenesis of the previous mutant strain and further selection in a 2-deoxyglucose medium (DILLON *et al*., 2011). Both mutant strains are kept under culture in the Enzymes and Biomass Laboratory (EBL) collection of University of Caxias do Sul (UCS). Therefore, this paper provides a systematic review of the literature regarding research on the *P. echinulatum* species.

2 Development

2.1 Systematic approach

Upon organizing the systematic review, an adaptation of the approach described by (UMAN, 2011) was adopted (Figure 1), containing the following steps: (*i*) formulation of an inquiry (Table 1); (*ii*) database selection; (*iii*) validation and filtering of search results according to the questions proposed previously. In this step, criteria such as PDF download and free access availability were taken into account, as well as fidelity to questions in step *i*; (*iv*) reading of the remaining articles, data organization and analysis, and Interpretation of the obtained results. For executing this workflow, the selected databases included: *Biblioteca Digital de Teses e Dissertações* (BDTD, http://bdtd.ibict.br/vufind/), PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), SciELO (https://www.scielo.org/) and ScienceDirect (https://www.sciencedirect.com/).

Figure 1 - Research workflow adopted for systematic review



Source: The authors.

Table 1 - Formulated questions and the proposed search strings for answering each of them (step *i*). Searches were conducted in the title and abstract search fields (english and portuguese)

Question	English search string	Portuguese search string
What are the objectives of research with *P. echinulatum*?	("*Penicillium echinulatum*" OR "*P. echinulatum*")	("*Penicillium echinulatum*" *OU* "*P. echinulatum*")
What are the important biotechnological applications related to *P. echinulatum*?	(("*Penicillium echinulatum*" OR "*P. echinulatum*") AND ("production" OR "secretion" OR "enzymes" OR "compounds"))	(("*Penicillium echinulatum*" *OU* "P. echinulatum") *E* ("*produção*" *OU* "*secreção*" *OU* "*enzimas*" *OU* "*compostos*"))
What strains of the fungus are used in research?	(("*Penicillium echinulatum*" OR "*P. echinulatum*") AND ("strain" OR "lineage"))	(("*Penicillium echinulatum*" *OU* "*P. echinulatum*") *E* ("*cepa*" *OU* "*linhagem*" *OU* "*estirpe*"))

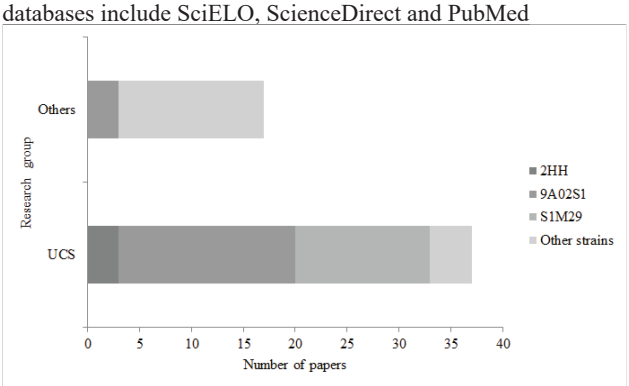
Source: Research data.

In the following sections the main findings of the selected papers have been compiled, providing a discussion on the main topics where pertinent. Due to a fraction of the articles being a result of the theses and dissertations, it was opted to include only a brief description of the latter as a separate section. Accordingly, the results from theses and dissertations have been covered by their respective articles.

2.2 *P. echinulatum* in biotechnology

As a result of the established workflow, 37 scientific articles were selected, as well as 12 theses and dissertations. Based on these papers found in the literature, it can be evidenced that the majority of works mentioning *P. echinulatum* were carried out by the same research group, UCS (Figure 2). In this institution, the enzymatic activity of different *P. echinulatum* strains has been the primary research focus with this fungus. Therefore, the first part of this review will explore the research efforts conducted by the biotechnology institute of this university, originating mostly from EBL-UCS.

Figure 2 - Number of research papers by institution of the first author, making use of different *P. echinulatum* strains. The papers considered were found with the following search string: ("*Penicillium echinulatum*" OR "*P. echinulatum*"). Investigated databases include SciELO, ScienceDirect and PubMed



Resource: Research data.

When it comes to the enzyme activity papers, diverse methods have been used with the intent of demonstrating the fungus biotechnological appliances, especially highlighting the potential for second generation ethanol production. The highest enzymatic activities obtained in published papers are represented in Tables 2 and 3, the first one containing studies carried out in solid state culture media, while the remainder with liquid media. The results shown for the activity of different enzymes correspond to the highest absolute values described in each study, without considering whether the data presented statistical difference against lower results of the papers. Besides that, it is important to mention that some of the articles presented end-results halting in an ascending tendency, suggesting that maximum values were not achieved.

2.3 Experiments conducted in solid state media

For solid state media, the highest xylanase and β-glucosidase activities were obtained from the S1M29 strain, making use of a mix between fodder radish cake (FRC) with wheat bran (WB) and a mix of elephant grass (*Pennisetum purpureum*) with wheat bran as inducer carbon sources, respectively (SCHOLL *et al*., 2015a; ZUKOVSKI *et al*., 2017). On the other hand, better results for filter paper activity (FPA) and endoglucanase activity were reached from the 9A02S1 strain (CAMASSOLA; DILLON, 2010). While FPA was better induced by a mix of sugarcane bagasse (SCB) pretreated by steam explosion with wheat bran, endoglucanase activity was enhanced by wheat bran exclusively. These were the highest results for solid state media and are highlighted in bold in Table 2. It is important to make clear that in each and every experiment a biomass pretreatment was employed.

Table 2 - Experiments conducted in solid state medium. Abbreviations are listed as follows: FPA (Filter Paper Activity), WB (wheat bran), PSCB (pretreated sugarcane bagasse), SCB (sugarcane bagasse), EG (Elephant Grass), FRC (Fodder Radish Cake), NI (not informed by the author)

Reference	Strain	Enzyme/Maximum Activity/Time			Major Inducer
CAMASSOLA; DILLON, 2007a	*P. echinulatum* 9A02S1	FPA	32.89 U gdm^{-1}	72 h	WB + PSCB
		Endoglucanase	282.36 U gdm^{-1}	96 h	
		β-glucosidase	58.95 U gdm^{-1}	96 h	
		Xylanases	9.95 U gdm^{-1}	72 h	
CAMASSOLA; DILLON, 2010	*P. echinulatum* 9A02S1	FPA	**45.82 U gdm^{-1}**	72 h	SCB + WB
		Endoglucanase	**290.47 U gdm^{-1}**	96 h	
		β-glucosidase	40.13 U gdm^{-1}	72 h	
		Xylanases	37.87 U gdm^{-1}	72 h	
SCHOLL *et al.*, 2015a	*P. echinulatum* S1M29	FPA	32.93 FPU g^{-1}	96 h	50% EG + 50% WB
		Endoglucanase	205.83 IU g^{-1}	120 h	
		β-glucosidase	**148.96 IU g^{-1}**	120 h	
		Xylanases	571.74 IU g^{-1}	120 h	
ZUKOVSKI *et al*., 2017	*P. echinulatum* S1M29	FPA	24.22 U g^{-1}	144 h	25% FRC + 75% WB
		Endoglucanase	210.5 U g^{-1}	144 h	
		β-glucosidase	22.62 U g^{-1}	96 h	
		Xylanases	**784.73 U g^{-1}**	120 h	
MENEGOL *et al.*, 2017	*P. echinulatum* 9A02S1	FPA	13.26 U g^{-1}	120 h	EG
		Endoglucanase	158.44 U g^{-1}	120 h	
		β-glucosidase	138.34 U g^{-1}	NI	
		Xylanases	372.62 U g^{-1}	NI	

Source: Research data.

In a study by Camassola and Dillon (2007a), the best results for FPA, endoglucanase, β-glucosidase and xylanases enzyme activity were obtained with the following inducer ratio respectively: 2pretreatedSCB(PSCB):8WB, 6PSCB:4WB, 2PSCB:8WB, and 2PSCB:8WB (CAMASSOLA; DILLON, 2007a). On the other hand, Camassola and Dillon (2010) also tested the possibility of employing sugarcane bagasse to replace a part of the wheat bran but this time without any pretreatment, obtaining the best results for the same enzymes with different biomass ratios: 6SCB:4WB, 0SCB:10WB, 6SCB:4WB and 4SCB:6WB (CAMASSOLA; DILLON, 2010). Both of these studies made use of *P. echinulatum* 9A02S1 and it is evident that the pretreatment did not influence most of the enzyme activity, since the best results found in each circumstance were apparently similar (Table 2). Nevertheless, the best xylanases activity stands out, being 9.95 U gdm^{-1} in the design with a pretreatment, and increased to 37.87 U gdm^{-1} without any pretreatment. This can be explained by the fact that once biomass undergoes a pretreatment process, the lignin and hemicellulose are significantly removed. This can hinder the production of xylanases (hemicellulase), given that hemicellulases are induced by the presence of hemicelluloses such as xylose (MENEGOL *et al.*, 2016a). Even though this mechanism is blocked, xylanase activity may still be initiated

by the presence of cellulose, due to similarities shared in gene regulatory elements controlling expression of the different enzymes such as the ACE2 transcription factor (ARO *et al.*, 2001).

Additionally, other studies have also tested the effect of different combinations and inducers concentrations, for example fodder radish and elephant grass (EG). In a study by Zukovski *et al.* (2017) the author obtained better enzyme activities by combining 1FRC:3WB rather than FRC alone (ZUKOVSKI *et al*., 2017). Furthermore, two papers have analyzed elephant grass as an inducer in solid state fermentation. In this respect, Scholl *et al*. (2015a) utilized EG pretreated by steam explosion at varying temperatures and duration. The author found better enzyme activity results after the following treatment specifications: 190ºC for 8 min for FPA, 200º C for 6 minutes for endoglucanase, and 180ºC for 6 minutes for both β-glucosidase and xylanases. Besides steam explosion, EG was also washed with distilled water afterwards in all cases (SCHOLL *et al.*, 2015a). At last, in a work by Menegol *et al*. (2017), EG was submitted to different pretreatments, obtaining the highest enzymatic activity of endoglucanase and FPA after sulfuric acid pretreatment, xylanases by combining 3EG:1WB without pretreatments, and β-glucosidase with untreated EG. These results indicate an influence caused by structural changes to the biomass, especially for the cellulolytic enzymes production, with the exception of β-glucosidases (MENEGOL *et al.*, 2017). In accordance with previous sugarcane bagasse papers, elephant grass displayed similar xylanase activity behavior, where a higher value is achieved by employing no pretreatment (CAMASSOLA; DILLON, 2010).

2.4 Experiments conducted in liquid state media

As for liquid state media, the highest enzymatic activities for FPA, endoglucanase, β-glucosidase and xylanase were obtained in a study by Reis *et al*. (2013), using cellulose as the inducer for the S1M29 strain (REIS *et al.*, 2013). As evidenced, various articles exhibit the cellulase production in submerged cultivations of this filamentous fungus, as represented in Table 3. In spite of this, it is difficult to directly compare the cellulase activities in these reports due to distinct assay methods as well as different research objectives. Nevertheless, specific activity values can be used as indicators for comparison.

Table 3 - Experiments conducted in liquid state medium. Abbreviations are listed as follows: FPA (filter paper activity), Cel (cellulose), Lac (lactose), NI (not informed by the author), WF (wheat flour), Sorb (sorbitol), SCB (sugarcane bagasse), Suc (sucrose), EG (elephant grass), SF (soy flour), YE (yeast extract), Glu (glucose), N/A (not applicable). *Maximum activity for different substrates according to specific activity in 96h

Reference	Strain	Enzyme/Maximum Activity/Time			Major Inducer
SEHNEM *et al*., 2006	*P. echinulatum* 9A02S1	FPA	-	168 h	25 % Cel +75% Lac
		β-glucosidase	-		100% Lac
CAMASSOLA; DILLON *et al.*, 2007b	*P. echinulatum* 2HH	FPA	-	144 h	Cel + 1 μ mol l^{-1} Caf
		β-glucosidase	-	192 h	
	P. echinulatum 9A02S1	FPA	-	192 h	Cel + 1 μ mol l^{-1} Caf
		β-glucosidase	-		Cel + 5 μ mol l^{-1} Teo
MARTINS *et al.*, 2008	*P. echinulatum* NI	FPA	0.27 U ml^{-1}	192 h	WF
		Endoglucanase	1.53 U ml^{-1}		
		β-glucosidase	0.31 U ml^{-1}		
		Xylanases	3.16 U ml^{-1}		
	T. reesei	FPA	110.2 U ml^{-1}	-	Cel
		Endoglucanase	766.67 U ml^{-1}		
		β-glucosidase	43.3 U ml^{-1}		
		Xylanases	1232.88 U ml^{-1}		
REIS *et al*., 2013	*P. echinulatum* S1M29	FPA	**8,3 U ml^{-1}**	144 h	Cel
		Endoglucanase	**37,3 U ml^{-1}**	120 h	
		β-glucosidase	**5,8 U ml^{-1}**	168 h	
		Xylanases	**177 U ml^{-1}**	120 h	
RITTER *et al*., 2013a	*P. echinulatum* 9A02S1	FPA	1.95 IU ml^{-1}	168 h	0.5% Sorb + 0.5% Cel after 24 h
		Endoglucanase	9.99 IU ml^{-1}	144 h	0.75% Sorb + 0.75% Cel after 12 h
		β-glucosidase	Low (<0,2 IU ml^{-1})	-	-
		Xylanases	-	96 h	0.75% Sorb + 0.25% Cel after 36 h

Reference	Strain	Enzyme/Maximum Activity/Time			Major Inducer
RITTER *et al*., 2013b	*P. echinulatum* 9A02S1	FPA	1.0 U ml^{-1}	120 h	0.75% Cel + 0.25% Sorb
		Endoglucanase	6.4 U ml^{-1}	100 h	
		Xylanases	5,5 U ml^{-1}	64 h	1% Cel
ZAMPIERI *et al.*, 2013	*P. echinulatum* 9A02S1	FPA	-	132 h	Cel
		Endoglucanase	-	108 h	
		β-glucosidase	-	108 h	
CAMASSOLA; DILLON, 2014	*P. echinulatum* 9A02S1	FPA	1.253 U ml^{-1}	144 h	SCB
		Endoglucanase	1.846 U ml^{-1}	144 h	
		β-glucosidase	0.288 U ml^{-1}	120 h	
		Xylanases	1.486 U ml^{-1}	72 h	
SCHNEIDER *et al*., 2014	*P. echinulatum* S1M29	FPA	0.69 IU ml^{-1}	120 h	SCB
		Endoglucanase	5.84 IU ml^{-1}	96 h	Cel
		β-glucosidase	1.43 IU ml^{-1}	96 h	Suc
		Xylanases	28.8 IU ml^{-1}	72 h	Cel
REIS *et al.*, 2015	*P. echinulatum* S1M29	FPA	1.45 U ml^{-1}	96 h	Cel
		Endoglucanase	6.7 U ml^{-1}		
		β-glucosidase	3.2 U ml^{-1}		
		Xylanases	30.5 U ml^{-1}		
SCHOLL *et al.*, 2015b	*P. echinulatum* S1M29	FPA	0.40 IU ml^{-1}	120 h	EG 10g l^{-1}
		Endoglucanase	3.43 IU ml^{-1}	120 h	
		β-glucosidase	1.39 IU ml^{-1}	96 h	
		Xylanases	9.97 IU ml^{-1}	120 h	
COSTA *et al.*, 2016	*P. echinulatum* S1M29	FPA	2.68 FPU ml^{-1}	144 h	SCB
		β-glucosidase	1.8 IU ml^{-1}		
		Xylanases	115.46 IU ml^{-1}		
		FPA	3.2 FPU ml^{-1}	144 h	SCB 10g l^{-1} + SF 5 g l^{-1} + YE 1 g l^{-1} + Suc 10 g l^{-1}
		β-glucosidase	3.1 IU ml^{-1}		
		Xylanases	112.2 IU ml^{-1}		
SCHNEIDER *et al.*, 2016	*P. echinulatum* 2HH	Endoglucanase	-	96 h*	Cel or SCB
		β-glucosidase	-		Glu
		Xylanases	-		SCB
		Pectinase	-		Cel or Glu
	P. echinulatum S1M29	Endoglucanase	-		Cel or SCB
		β-glucosidase	-		Glu
		Xylanases	-		Cel
		Pectinase	-		Cel
MENEGOL *et al.*, 2016a	*P. echinulatum* 9A02S1	FPA	0.17 IU ml^{-1}	120 h	EG
		Endoglucanase	1.98 IU ml^{-1}	120 h	
		β-glucosidase	0.87 IU ml^{-1}	120 h	
		Xylanases	1.21 IU ml^{-1}	96 h	
SCHNEIDER *et al*., 2018	*P. echinulatum* S1M29	FPA	0.23 U ml^{-1}	120 h	SCB
		Endoglucanase	0.69 U ml^{-1}	96 h	SCB
		β-glucosidase	0.05 U ml^{-1}	120 h	Glu
		Xylanases	2.81 U ml^{-1}	120 h	SCB
	P. echinulatum 2HH	FPA	0.04 U ml^{-1}	48 h	SCB
		Endoglucanase	0.26 U ml^{-1}	48 h	SCB
		β-glucosidase	0.06 U ml^{-1}	120 h	Cel
		Xylanases	0.00 U ml^{-1}	N/A	N/A

Source: Research data.

Still on the work carried out by Reis *et al*. (2013), the maximum enzyme activity for FPA, endoglucanase and xylanase were obtained in a fed-batch culture with 40 g l^{-1} of cellulose, while for β-glucosidases both batch and fed-batch experiments using 60 g l^{-1} of cellulose. The fed-batch technique has shown to be more adequate, probably due to the low levels of glucose kept in the culture. In contrast, when the carbon source concentration in the culture is found above a certain threshold, cellulases production can be suppressed, which might happen in batch processes (REIS *et al*., 2013). Trying to further optimize *P. echinulatum* S1M29 enzyme production, Reis *et al*. (2015) evaluated

different mineral and urea concentrations in the culture medium composition. As a result, the increase in some salts concentration promoted improved enzymes activity in relation to the standard medium. According to the authors, an optimal formulation contains: KH_2PO_4 (2.0 g l^{-1}), $(NH_4)_2SO_4$ (1.4 g l^{-1}), $MgSO_4.7H_2O$ (0.375 g l^{-1}), $CaCl_2$ (0.375 g l^{-1}), and urea (0.525 g l^{-1}) (REIS *et al*., 2015).

Considering the carbon source used in the experiments, several additives have been study targets. Taking that into account, Camassola and Dillon (2007b) examined the effect of aminophylline, caffeine and theophylline on cellulases production by *P. echinulatum* strains 2HH and 9A02S1. For the 2HH strain, better results were observed when the cellulose medium was supplemented with 1 μ mol l^{-1} of caffeine for both FPA and β-glucosidases. It is notable how caffeine had a positive effect in comparison to the non-supplemented cellulose medium, while both aminophylline and theophylline had a negative effect on overall production. As for the 9A02S1 strain, besides the caffeine positive effect, theophylline supplemented media also showed enhanced enzyme activity for both FPA and β-glucosidases (CAMASSOLA; DILLON *et al*., 2007b). Furthermore, a study by Sehnem *et al*. (2006) analyzed the lactose potential as an inducer for cellulases production of the 9A02S1 strain. In this experiment, the author tested different lactose concentrations in conjunction with cellulose. As a result, FPA was consistent from 0-75% lactose, reaching values around 1.5 FPU ml^{-1} after 168 hours. The use of lactose by itself displayed drastically low results for FPA, whereas this same condition exhibited the highest β-glucosidases result (SEHNEM *et al*., 2006).

Elephant grass has also been highlighted as a promising prospect for inducer biomass. In that respect, Scholl *et al*. (2015b) investigated multiple steam explosion pretreatment variations in order to improve cellulases and xylanases production by *P. echinulatum* S1M29. Making use of EG as a carbon source, the pretreatment at 190 ºC for 8 minutes showed positive results for FPA, and the same temperature for 6 minutes for endoglucanases and β-glucosidases. Moreover, xylanases activity was improved following a 200 ºC pretreatment for 10 minutes. Interestingly, the best results for FPA and endoglucanases were obtained when the biomass was washed after the pretreatment, while for better enzyme yields of β-glucosidases and xylanases, the biomass did not undergo a washing step (SCHOLL *et al*., 2015b). Even more, this time making use of *P. echinulatum* 9A02S1, Menegol *et al*. (2016a) submitted EG to other pretreatments. As the outcome, better FPA, endoglucanase, and β-glucosidase enzymatic activity was obtained with biomass that had previously been treated with sulfuric acid (at 10%, 10%, and 5%, respectively). On the other hand, xylanase activity proved higher when the biomass was autoclaved (MENEGOL *et al*., 2016a).

Another alternative biomass that has been thoroughly tested is sugarcane bagasse. From this perspective, a study by Camassola and Dillon (2014) analyzed different biomass pretreatments for subsequent enzyme production by *P. echinulatum* 9A02S1. Sodium hydroxide, hydrogen peroxide, anthraquinone, and ethylenediaminetetraacetic acid solutions were employed in the sugarcane bagasse delignification prior to fermentation. As a result, FPA, endoglucanase, and β-glucosidase activities were higher in pretreated conditions instead of the untreated SCB and Cellulose (Cel) control groups (CAMASSOLA; DILLON, 2014). On the other hand, xylanase presented higher activity for Cel, with similar results as to untreated SCB. Furthermore, Costa *et al*. (2016) conducted an extensive multilayered study to evaluate *P. echinulatum* S1M29 enzyme production. In a first stage, three sugarcane bagasse pretreatment options were assessed: pressurized hot water, steam explosion followed by alkaline delignification, and steam explosion. Even though not much difference was observed among them, steam explosion by itself presented the best results for FPA, β-glucosidases and xylanases. Carrying on, SCB pretreated by steam explosion was used at a fixed concentration 10 g l^{-1} in combination with other carbon sources, including soybean flour (SF), wheat flour (WF), sucrose (Suc), yeast extract (YE), and salt solution (SS). Considering that, three media to enhance each of the enzyme groups were proposed. The analysis showed that the medium destined to enhance β-glucosidase activity provided the best substrate for all the tested enzymes, being composed of 10 g l^{-1} of steam-exploded SCB, 5.0 g l^{-1} of SF, 1.0 g l^{-1} of YE, 10.0 g l^{-1} of Suc, and 50 ml l^{-1} of SS (COSTA *et al*., 2016).

2.5 Proteome analyses

Furthermore, other studies (not included in tables 2 and 3) have explored the enzyme yield produced by different lineages of *P. echinulatum*. Ribeiro *et al*. (2012), for instance, characterized the *P. echinulatum* 9A02S1 secretome, revealing an enzyme machinery composed mostly by cellulases, in particular endoglucanases, cellobiohydrolases and *β*-glucosidases. Among the carbohydrate-active enzymes, diverse glycoside hydrolase (GH) families were identified: endoglucanases GH5, GH7, GH6, GH12, GH17 and GH61; *β*-glucosidases GH3; xylanases GH10 and GH11; debranching hemicellulases from GH43, GH62 and carbohydrate esterase family CE2; and pectinases from GH28 (RIBEIRO *et al*., 2012).

Additionally, *P. echinulatum* S1M29 has also been well studied. In order to characterize the enzyme complex obtained in sugarcane bagasse submerged culture, Costa *et al*. (2016) identified the corresponding protein families through mass spectrometry. Accordingly, it was found that the majority of the enzymes were: cellobiohydrolases of the GH6 and GH7 families, endoglucanases of the GH5 family, and endo-1,4-β-xylanases of the GH10 family (COSTA *et al*., 2016). In addition, a study by Schneider *et al*. (2016)

examined the wild (2HH) strain performance in comparison to the mutant (S1M29). The mutant demonstrated a more efficient enzymatic repertoire for lignocellulosic biomass degradation. Therefore, a proteome characterization was carried out, revealing a secretome total of 165 proteins, of which one third belongs to CAZy families of GH3, GH5, GH17, GH43, and GH72. From those, 50% are cellulases, hemicellulases and other enzymes related to plant cell wall degradation (SCHNEIDER *et al*., 2016).

2.6 Second generation ethanol production

Some of the related works have managed to investigate the efficacy of *P. echinulatum* enzyme complex applied in 2G Ethanol Production. Additionally, Menegol *et al*. (2014; 2016b) carried out two studies making use of elephant grass as an inducer for enzyme production. The first one examined the enzyme complex of *P. echinulatum* 9A02S1, produced both in solid and liquid state culture. Here, diverse elephant grass pretreatments were also tested, revealing that sodium hydroxide employment promotes the glucose release, reducing sugars and lignin, therefore improving efficiency of the posterior enzymatic hydrolysis. Another important result indicates that solid state culture tends to be more adequate for cellulase production, particularly endoglucanases (MENEGOL *et al*., 2014). In their next work, the authors analyzed the effect of a biomass treatment in parallel with enzymatic hydrolysis. With that intent, ball milling was performed in a rotating hydrolysis reactor with elephant grass and the addition of *P. echinulatum* S1M29 enzymes produced in solid state culture. Posterior ethanol production doubled by implementing this alternative condition in comparison with the use of a static bioreactor (MENEGOL *et al*., 2016b).

Differing from the previous analyses, Aver *et al*. (2014) looked at sugarcane bagasse pretreatments for posterior hydrolysis by *P. echinulatum* S1M29 enzymes produced in solid state culture. Considering this, the researchers pretreated the biomass with different concentrations of ionic liquids at two temperatures (80 and 120 °C), which was then subjected to hydrolysis. Afterwards, the amount of glucose and reducing sugars was measured to determine efficiency. Furthermore, scanning electron microscopy was performed to identify the pretreatments effect on the lignocellulosic material morphology. Overall, administration of 1-butyl-3-methylimidazolium acetate at 120 °C was found as the best pretreatment, showing biomass surface alteration and obtaining the highest yields (AVER *et al*., 2014).

2.7 Genetic and molecular studies

Besides enzyme activity assessments, *P. echinulatum* has, throughout its history, undergone a number of works examining its genetic and molecular characteristics. Concerning that, Rubini *et al.* (2010) performed the first isolation, cloning and expression description of a *P. echinulatum* 9A02S1 cellulase gene. Accordingly, the identified egl1 cDNA (*Pe-egl*1) encodes for a putative endoglucanase, which was then cloned inside a heterologous expression system with a yeast (*Pichia pastoris*) as a host. The recombinant EGL1 enzyme was identified as a member of the GH5 family with high thermostability and activity over a wide pH range. What is more, a possible double function was also suggested by the authors, whereupon the enzyme acts as both endo- and exoglucanase (RUBINI *et al*., 2010). In another work, looking to support forthcoming gene expression research using quantitative reverse-transcription polymerase chain reaction (qRT-PCR), Zampieri *et al*. (2014) examined and validated *P. echinulatum* S1M29 internal control genes. In accord with other filamentous fungi, the *β*-actin gene (*actb*) was identified as a reference gene, being recommended as an endogenous control due to its stable and continuous expression (ZAMPIERI *et al*., 2014).

Continuing works with this fungus, Basso *et al*. (2014) evaluated the viability of inducer biomass substitution from cellulose to elephant grass for second generation ethanol production. In their work they made use of 85 genotypes of elephant grass as substrate for cellulase production by *P. echinulatum* 9A02S1. The best results for *β*-glucosidase and xylanase activities were obtained from genotypes IJ7125 and Mercker 86 Mexico, respectively, while endoglucanase and filter paper activity from IJ7127. Moreover, the results indicate a high potential for replacement of the traditional biomass with alternative sources, especially since some tests notably exceed the enzymatic activity of the control, reaching up to an increase four times greater (BASSO *et al*., 2014).

A notable development was the execution of a protoplast fusion between *P. echinulatum* and *T. harzianum*, resulting in selected lineages that outperformed parental strains in FPA and β-glucosidases production. Overall, the obtained fusants emerge as better enzyme producers, which demonstrates how this fusion technique in conjunction with lineage selection constitutes an efficient methodology design for microorganisms genetic engineering (DILLON *et al*., 2008).

2.8 Theses and dissertations

Besides the previously mentioned articles, the scientific production related to the 2HH isolate and other derived strains also includes theses and dissertations (Tables 4 and 5). Between 2007 and 2017, a total of 12 open access works were published, including eight masters and four doctorates, with the highest number of publications per year reaching three works in 2014. In total, 10 were fulfilled for the biotechnology postgraduate programme of UCS and the remainder at the University of Brasília for the molecular biology and molecular pathology postgraduate programmes.

Table 4 - Master's degrees related to *P. echinulatum*. Abbreviation: PGP (Postgraduate programme)

Reference	Title	PGP
SOUZA, 2015	Fusão de protoplastos entre *Penicillium echinulatum* e *Trichoderma harzianum* para obtenção de variabilidade visando a produção de celulases	Biotechnology
RITTER, 2014	Produção de celulases e xilanases por *Penicillium echinulatum* em processo submerso utilizando biorreatores com agitação mecânica e airlift de circulação interna	Biotechnology
ZAMPIERI, 2015	Expressão do complexo celulolítico em *Penicillium echinulatum*	Biotechnology
MATOS, 2012	O sistema celulolítico de *Penicillium echinulatum* : análise da ultraestrutura miceliana e influência de moduladores epigenéticos	Molecular Pathology
REIS, 2014	Produção de celulases e xilanases por *Penicillium echinulatum* em biorreator com agitação mecânica	Biotechnology
SCHNEIDER, 2015	Secretômica e atividades enzimáticas da linhagem selvagem 2HH e do mutante S1M29 de *Penicillium echinulatum*	Biotechnology
ZUKOVSKI, 2015	Produção de celulases e xilanases pelo fungo *Penicillium echinulatum* empregando resíduos da extração de lipídios de nabo forrageiro	Biotechnology
SCHOLL, 2015c	Capim-elefante pré-tratado por explosão a vapor como matéria-prima para produção de enzimas, hidrólise enzimática e fermentação alcoólica	Biotechnology

Source: Research data.

Table 5 - Ph.D degrees related to *P. echinulatum*. Abbreviation: PGP (Postgraduate programme)

Reference	Title	PGP
RUBINI, 2009	Clonagem, expressão heteróloga e caracterização funcional de uma endoglicanase de *Penicillium echinulatum*	Molecular Biology
RITTER, 2015	Efeito da adsorção e filtração na produção de celulases e xilanases	Biotechnology
ZAMPIERI, 2016	Expressão gênica e atividades de celulases, ß-glicosidases, xilanases e swoleninas de *Penicillium echinulatum* S1M29	Biotechnology
REIS, 2017	Estratégias para incremento das atividades de celulases e xilanases por *Penicillium echinulatum* S1M29 em cultivo submerso	Biotechnology

Source: Research data.

2.9 Other *P. echinulatum* studies

Apart from the lignocellulolytic focus, *P. echinulatum* has also appeared in a variety of different studies. Whereas the strains used in investigations by UCS are all derived from the 2HH wild-type isolate, there have been several works from other research groups that report isolation of the same species from a number of different sources, including red alga (LI *et al.*, 2014), blue grape mold (KIM *et al.*, 2007), dry-cured ham (NÚÑEZ *et al*., 1996), soil samples (ANDERSON *et al*., 1988) cheese (LUND; FILTENBORG; FRISVAD, 1995) and even subglacial ice (SONJAK; FRISVAD; GUNDE-CIMERMAN, 2006). Even though this fungus is observed in a broad diversity of environments, it has mostly been described as a food-borne fungi, and in these cases being portrayed as a toxigenic species. In that context, Lund *et al*. (1995) analyzed the mycoflora of industrialized cheese (SONJAK; FRISVAD; GUNDE-CIMERMAN, 2006). The authors found *P. echinulatum* present in a few packaged cheeses from different countries, producing penechins (more currently known as arisugacins) as secondary metabolites, which are acetylcholinesterase inhibitors. In agreement with the previous paper, Sonjak *et al*. (2006) and Li *et al*. (2014) also report production of arisugacins by *P. echinulatum*, this time isolated from subglacial ice and marine red alga *Chondrus ocellatus*, respectively (LI *et al*., 2014; SONJAK; FRISVAD; GUNDE-CIMERMAN, 2006). In their combined findings they also state production of the following extraolites: palitantin, territrem B and C, cyclopeptin, dehydrocyclopeptin, cyclopenin, cyclopenol, viridicatin, viridicatol. Nevertheless, secondary metabolite profile variation is expected between studies due to substrate composition variation (NÚÑEZ *et al*., 2007).

While older articles seem to base species level identification on morphological observations, dichotomous keys and eventually secondary metabolite profile analysis, more recent research usually include a molecular method in conjunction. Case in point, Li *et al*. (2014) analyzed the Internal Transcribed Spacer region of the rDNA in addition to morphological observations in order to classify an algicolous fungi isolate as *P. echinulatum*, while Kim *et al*. (2007) compared *β*-tubulin gene sequences of their grape mold isolate with references deposited in GenBank. In addition, some of these articles even stress the importance of including a molecular approach to species level identification, given the diversity of the *Penicillium* genus combined with high variability within each species (LI *et al*., 2014; KIM *et al*., 2007).

Even more on this topic, Kim *et al*. (2012) performed a chemotaxonomy analysis, together with an antioxidant activity evaluation, in order to identify and classify isolates of the *Penicillium* genus. For classification according to metabolites, the authors used liquid chromatography-electrospray ionization ion trap-mass spectrometry, gas chromatography-ion trap-mass spectrometry and a multivariate statistical

analysis. The dendrogram generated from the results of the first analysis was similar to the dendrogram with the internal transcribed spacer (ITS) marker, supporting the accuracy of the chemotaxonomy approach results. In general, the procedure indicated the strains in concern to be representative of four taxonomic species: *P. echinulatum*, *P. expansum*, *P. solitum*, and *P. oxalicum*. The use of alternative methodologies in taxonomy, such as chemotaxonomy, and molecular and physiological analyses make up essential tools in classification, not only for *Penicillium* species, but also applied to other filamentous fungi. In conclusion, the use of classical taxonomy based on morphological aspects and teleomorphic states presents difficulties, considering that these characteristics are not well defined for the *Penicillium* genus (HETTICK *et al*., 2008; KIM; PARK; LEE, 2012).

3 Conclusion

This literature review of *P. echinulatum* allowed further elucidation of research objectives surrounding this fungal species, namely: the hyper-productive lineages selection, the understanding of the enzymatic repertoire and enhancement of biotechnological processes. Besides these, there have been other less pronounced efforts outside UCS and with distinct purposes. Nevertheless, the major biotechnological application in studies with *P. echinulatum* involves obtention of viable plant-biomass-degrading enzyme cocktails, economically feasible from a marketplace perspective. Furthermore, the three lineages most used in research to achieve those goals are strains 2HH, 9A02S1 and S1M29, from which the latter has proven to be most efficient overall.

Many other papers have reported the isolation of *P. echinulatum* from a variety of different places (red alga, grape mold, ham, soil, cheese and subglacial ice), suggesting a wide range of habitat tolerance. Additionally, a lack of an integrated taxonomic identification method, making use of molecular, morphological and chemotaxonomic analyses was observed in the taxon classification isolated in UCS. The use of multiple approaches in species classification, as described in more recent studies, can provide a more complete understanding of the organism in question. This could possibly allow for the recognition of further biotechnological applications besides lignocellulolytic enzymes production.

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