Research, Society and Development, v. 11, n. 10, e136111032583, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i10.32583

Fungal amylases applied to the sweet potato starch for bioethanol production

Amilases fúngicas aplicadas em fécula de batata-doce para produção de bioetanol

Amilasas fúngicas aplicadas al almidón de batata para la producción de bioetanol

Received: 07/06/2022 | Reviewed: 07/15/2022 | Accept: 07/17/2022 | Published: 07/25/2022

Tiago Carnevalle Romão
ORCID: https://orcid.org/0000-0003-2821-779X
Instituto Federal Goiano, Brazil
E-mail: tiago.romao@ifgoiano.edu.br
Antonio Carlos Pereira de Menezes Filho
ORCID: https://orcid.org/0000-0003-3443-4205
Instituto Federal Goiano, Brazil
E-mail: astronomoamadorgoias@gmail.com
Aristeu Gomes Tininis
ORCID: https://orcid.org/0000-0002-1731-7149
Instituto Federal de São Paulo, Brazil
E-mail: aristeu@ifsp.edu.br
Marilene Silva Oliveira
ORCID: https://orcid.org/0000-0002-5801-2288
Instituto Federal Goiano, Brazil
E-mail: marilenes36@gmail.com
Lidiane Gaspareto Felippe
ORCID: https://orcid.org/0000-0003-3797-3677
Universidade Estadual Paulista, Brazil
E-mail: lidiane.felippe@unesp.br
Carlos Frederico de Souza Castro
ORCID: https://orcid.org/0000-0002-9273-7266
Instituto Federal Goiano, Brazil
E-mail: carlos.castro@ifgoiano.edu.br
Paula Benevides de Morais
ORCID: https://orcid.org/0000-0002-5050-5766
Universidade Federal do Tocantins, Brazil
E-mail: moraispb@mail.uft.edu.br

Abstract
Bioethanol is a sustainable energy source to help reducing the emission of pollutants into the global environment. In order to cope with that, the ethanol production technologies and use of efficient and low-cost substrates are developed. The objective of this research was to evaluate fungal amylases in sweet potato starch for bioethanol production by *Saccharomyces cerevisiae* in the laboratory. *Endomelanconiopsis endophytica* (1.40 U/mL), *Neopestalotiopsis cubana* (1.67 U/mL) and *Fusarium pseudocircinatum* (1.11 U/mL) with high enzymatic activities were selected and their amylases were tested for activity on sweet potato starch for bioethanol production. Simultaneous saccharification and fermentation was performed at 30° C and pH = 5.0. 17.3 - 88.1 (%) of bioethanol that was produced and compared to the expected theoretical yield. Therefore, amylases from these fungi simultaneously inserted on sweet potato starch and *S. cerevisiae* are potentially useful for bioethanol production.
Keywords: Amylases; Sweet potato; Bioethanol production; *E. endophytica; N. cubana; F. pseudocircinatum.*

Resumo
O bioetanol é uma fonte energética sustentável para auxiliar a redução de emissão de poluentes no meio ambiente global. Para isso, tecnologias de produção de etanol e uso de substratos eficientes e de baixos custos são desenvolvidos. O objetivo desta pesquisa foi avaliar amilases fúngicas em fécula de batata-doce para produção de bioetanol pela *Saccharomyces cerevisiae* em laboratório. *Endomelanconiopsis endophytica* (1,40 U/mL), *Neopestalotiopsis cubana* (1,67 U/mL) e *Fusarium pseudocircinatum* (1,11 U/mL) com altas atividades enzimáticas foram selecionadas e suas amilases foram testadas para atividade em fécula de batata-doce para produção de bioetanol. A sacarificação e fermentação simultâneas foram realizadas a temperatura de 30 °C e pH = 5,0. 17,3 - 88,1 (%) de bioetanol foram produzidos em relação ao rendimento teórico esperado. Portanto, as amilases desses fungos inseridas simultaneamente sobre a fécula da batata-doce e *S. cerevisiae* são potencialmente úteis para a produção de bioetanol.
Palavras-chave: Amilases; Batata-doce; Produção de bioethanol; *E. endophytica; N. cubana; F. pseudocircinatum.*

Resumen
El bioetanol es una fuente de energía sostenible que contribuye a reducir las emisiones contaminantes en el medio ambiente. Para ello, se desarrollan tecnologías para la producción de etanol y el uso de sustratos eficientes y de bajo coste. El objetivo de esta investigación fue evaluar las amilasas fúngicas en el almidón de batata para la producción de bioetanol por *Saccharomyces cerevisiae* en laboratorio. Se seleccionaron *Endomelanconiopsis endophytica* (1,40 U/mL), *Neopestalotiopsis cubana* (1,67 U/mL) y *Fusarium pseudocircinatum* (1,11 U/mL) con actividades enzimáticas altas y se probaron sus amilasas asociadas al almidón de batata para la producción de bioetanol. La sacarificación y la fermentación simultáneas se realizaron a 30° C y pH = 5,0. Se produjo un 17,3 - 88,1 (%) de bioetanol, en comparación con el rendimiento esperado. Por lo tanto, las amilasas de estos hongos insertadas simultáneamente en el almidón de la batata y en *S. cerevisiae* son potencialmente útiles para la producción de bioetanol.
Palabras clave: Amilasas; Batata; Producción de bioetanol; *E. endophytica; N. cubana; F. pseudocircinatum.*

1. Introduction

In the future, non-renewable energy sources such as crude oil and its derivatives, natural gas and coal, which together account for 81.1% of the world's energy demand (International Energy Agency, 2021) will be of little use. Thus, the global community aims to reduce the dependence on fossil fuels and to find sustainable forms of energy, such as biofuels (Aditiya et al., 2016).

In Brasil, the contribution of renewable energy matrices is among the largest in the world (48.4%), with 33.1% coming from biomass (Ministério das Minas e Energia, 2021). In 2020, the sectors that presented the highest use of renewable sources were industry and transportation. The industrial sector presented renewability of 63% of its energy matrix. The fraction of renewable energy used in Brazilian transportation is currently 25%, with ethanol accounting for about 19.3% of fuel consumption (Ministério das Minas e Energia, 2021). These indices, which place Brasil in a prominent position on the world scene in the use of biomass as an alternative energy matrix with the use of fossil fuels, justify the need to seek the commercial use of bioproducts derived from plant material which are rich in carbohydrates for the production of biofuels. The Brazilian technology for producing bioethanol from sugarcane is one of the most successful and efficient worldwide. It's uses low-cost substrates, such as lignocellulosic material, which results in more competitive biofuels (Gonçalves et al., 2013). Second generation ethanol is derived from cellulosic or starchy biomass, rich in carbohydrates that can be hydrolyzed into glucose (Machado & Abreu, 2006), and it represents attractive renewable energy sources that can contribute widely to the implementation of sustainable and less polluting use of energy matrices.

In China, sweet potato (*Ipomea batatas* L.) was chosen as the main vegetable for bioethanol production, since the country produces about 92 million tons, i.e., more than 58% of the world's production. Asia with 66% of this total, followed by Africa (28.3%) and the Americas (4.6%). Brazil ranks 16th among the largest producers of sweet potato, with 805,4 tons (Empresa Brasileira de Pesquisa Agropecuária [Embrapa], 2021).

In this context, among biofuels, bioethanol is one of the sustainable alternatives for the global environmental issue. Besides being environmentally friendly, it can replace gasoline (Gronchi et al, 2019). Aiming to produce bioethanol on a laboratory or industrial scale, starch (dry) or even fresh sweet potato can be used by simultaneous saccharification and fermentation (SSF) by *Saccharomyces cerevisiae*, however, this yeast is not able to degrade starch, so it is necessary to add enzymes wich are capable to hydrolyze starch in order to use this carbon source (Zhang et al., 2011).

Microorganisms producing α-amylases are desirable sources and of a wide interest because these enzymes exhibit structural and functional stability (Kumar et al., 2016). Among different microbial organisms, such as fungi and bacteria, few are capable of producing α-amylases, *e.g.*, *Lipomyces konenkoae*, *Streptomyces bovis*, and glucoamylases, *e.g.*, *Rhizopus oryzae*, *Aspergillus awamori*, and *Aspergillus tubingensis*, as well as they are efficient in degrading starch (Favaro et al., 2015). Among the micro-organisms, fungi are the most responsive to industrial demands (Beltagy et al., 2022).

Amylases are hydrolases that catalyze the hydrolysis of starch synergistically, producing dextrins and other smaller polysaccharides (Van Der Maarel et al., 2002). Thus, α-amylase hydrolyzes internal α-1,4 bonds of the polymer producing linear or branched oligosaccharides. In turn, β-amylases, glucoamylases break α-1,4 or α-1,6 bonds of non-reducing terminals of the amylose and the amylopectin chain; and de-ramifying enzymes hydrolyze α-1,6 bonds in the branches of the polysaccharide (Astolfi, 2019). Enzymatic hydrolysis of starch receives attention by researchers and the biofuels industry, as they can reduce energy consumption and simplify production (Favaro et al., 2015)

In this context, in order to produce ethanol at the laboratory, in a pilot or industrial scale from sweet potato, starch (dry) or even fresh sweet potato can be used by simultaneous saccharification and fermentation (SSF) by *Saccharomyces cerevisiae* (Zhang et al., 2011). SSF is an operational process that, in a single step, combines saccharification and fermentation. In this assay, glucose monomers released by enzymatic hydrolysis of starch or lignocellulosic residues are rapidly converted into ethanol (Souza Filho et al., 2016; Schweinberger et al, 2016; Schweinberger, 2016). However, there are difficulties in optimizing the temperature and pH parameters for enzymes and microorganisms concomitantly (Schweinberger, 2016).

Aiming to try to circumvent these possible problems and to study the biotechnological potential of amylases from fungi in the digestive tract of plant material fragmenting insects, we tested three fungal strains: *Endomelanconiopsis endophytica*, *Neopestalotiopsis cubana* and *Fusarium pseudocircinatum* that produced the highest levels of amylases with potential to saccharify raw sweet potato starch. Thus, portions of raw starch were treated with enzymes from each of the selected fungi and *Saccharomyces cerevisiae* for efficient bioethanol production.

2. Methodology

2.1 Fungal selection

Thirty-six fungi from the Carlos Rosa Collection (CRC) of the Universidade Federal do Tocantins, Palmas-TO, Brasil, were evaluated for their potential to produce amylases. The three strains with higher amylolytic enzyme activity were selected and tested for bioethanol production.

2.2 Growth media

The selected fungi were reactivated in Petri plates containing microbiological medium Merck® potato-dextrose agar (PDA) prepared at 1% (w/v) concentration. The fungi were then incubated in biochemical oxygen demand (BOD) - TECNAL 371 adjusted at 26 ºC for up to 10 days for growth. Subsequently, a disk of each fungal isolate, approximately 1 cm in diameter, was transferred to a 25 mL flask containing 3% malt extract solution on a rotary shaker (100 rpm) for 3 days at room temperature for mycelium growth, and then small aliquots of mycelium were stored in Eppendorf tubes (1.5 mL) and frozen at -20º C.

2.3 Fungal enzymatic biosynthesis

A mineral solution following that proposed by Papanikolaou and Aggelis, (2002) was prepared and enriched with sweet potato starch 1% (w/v). A disc of approximately 1 cm diameter was taken from the cultured microbiological medium (PDA) of each strain and inoculated into Erlenmayer flasks (125 mL) containing enriched mineral solution. Then, the flasks were placed under agitation on SL-180/DT SOLAB® shaker table (100 rpm) for 5 days for biosynthesis of amylolytic enzyme extract. In this study, the amylolytic enzyme activity and characterization assays were performed in triplicate.

2.4 Amilolytic enzyme assay

The amylolytic enzymatic extracts of each strain were submitted to the enzymatic activity assay, proposed by Ghose, (1987) adapted, to choose three fungal strains with higher amylolytic enzymatic activities.

The amylolytic activities were determined by mixing 0.5 mL of amylases from each fungal strain with 0.5 mL of 1% (w/v) substrate solution (0.5 g of sweet potato starch mixed to 50 mL citrate buffer pH= 4.8). Then the solution was heated in a water bath at 50 °C for 30 min. The amount of reducing sugar released was measured using 3,5-dinitrosalicylic acid (DNS) reagent (Miller, 1959). The potential (volumetric) amylolytic enzyme activity was calculated as micromol per min (µmol min-1) of reducing sugar in the enzyme solution (U/mL) according to equation 1 (Ghose, 1987).

$$[Activity](U/mL) = [glucose](mg/mL)\ 0{,}37 \qquad \text{Eq. 1}$$

Where: 0.37= estimated glucose content released upon hydrolysis of 1 U.

Total protein concentration was determined by the ultraviolet absorbance assay (Layne, 1957; Aitken & Learmonth, 2001).

Equation 2 (Layne, 1957) for determining approximate total protein concentration using ultraviolet absorbance is as follows:

$$[protein](mg/mL) = (A_{280})\ 1{,}55 - (A_{260})\ 0{,}76 \qquad \text{Eq 2}$$

2.5 Strain identity

Mycelium samples from the 3 strains with best amylolytic enzyme activity were sent for molecular identification, amplification, and gene sequencing to the laboratory of Applied Molecular Biology at Instituto Biológico, São Paulo-SP, Brasil (Romão et al., 2024).

2.6 Sweet potato starch

The sweet potato starch was obtained by donation of a sealed 400 g package with nutritional label stating 85% starch, by a local merchant.

2.7 Enzyme characterization - pH and thermal stability

Portions of sweet potato starch were added to flasks containing citrate buffer at pH= 4.0; 4.5; 5.0; 5.5; 6.0; 6.5 to form 1% (w/v) substrate solutions. Then 0.5 mL of amylases from each of the fungi were added separately to 0.5 mL of substrate solution at each pH and the release of reducing sugars (Miller, 1959) was quantified at each pH for determination of amylolytic activity as a function of pH.

Thermal stability of the amylases was verified by standardized assay of enzyme activity at temperatures ranging from 20 °C to 90 °C. Samples (50 mL) of enzyme solution were incubated in an ultratermostatic bath (SL 152 SOLAB®) for 1 hour (h) at temperatures of 20, 30, 40, 50, 60, 70, 80 and 90 °C, and aliquots (0.5 mL) were taken at each temperature to measure the amylolytic enzymatic activity as previously described according to Ghose (1987) and adapted from it

2.8 Simultaneous saccharification fermentation assay

Triplicate solutions at 1% (w/v) were produced by mixing 0.5 g of sweet potato starch added to 50 mL of amylolytic solution in citrate buffer (pH = 5.0) from each of the three fungal strains and 0.5 g of FERMEL® *Saccharomyces cerevisiae* pellets, used in ethanol production industries, to perform the simultaneous saccharification and fermentation (SSF) assay. The

bioreactors - Erlenmeyer flasks (125 mL) - were sealed with cotton stoppers and then incubated in an ultrathin bath (SL 152 SOLAB®) adjusted to 30°C for 48 hours (h).

The standardized assay to verify SSF and consequent potential bioethanol production was performed by withdrawing 1 mL aliquots from samples incubated at the following time intervals (t): 0, 1, 2, 4, 6, 12, 24, and 48 h. Then, verifying reducing sugars (Miller, 1959).

2.9 Determination of ethanol concentration

Triplicate aliquots (1 mL) of SSF solution from each sample were withdrawn at each time interval (t) and placed in Eppendorf tubes (1.5 mL), then frozen at (-20 ºC) and sent to Instituto Federal de São Paulo, Matão, São Paulo, Brasil, for quantification of ethanol in Trace GC Ultra Thermo Scientific gas chromatography equipment with FID detector and TRIPLUS Headspace automatic injector, Agilent DB-5 column (30 mm 30.25 mm).

The assay was performed by injecting 1 µL of the sample, keeping the column at 35 °C for 7 minutes. Then a heating ramp of 15 °C/min was performed until 220° C was reached, and it was kept at 220° C for 1 minute. The injector temperature was maintained at 250 °C. After the gas chromatographic assay, the concentrations (% v/v) of bioethanol were determined using the standard calibration curve equation.

2.10 Statistical analysis and caculation

The results obtained were expressed as means and standard deviation, and the bioethanol means were compared by Tukey's test at 95% confidence level ($p < 0.05$).

3. Results and Discussion

3.1 Fungi identification

The filamentous fungi identified and analyzed in this study are: *Endomelanconiopsis endophytica*, *Neopestalotiopsis cubana* and *Fusarium pseudocircinatum.* The molecular sequences and morphological structures were described by Romão et al. (2024).

Using the Web of Science™ and PubMed® databases, as well as using as keywords "fungal name" and "amylases" and "biothanol", no scientific articles were found in the last 5 years that reported application of the fungi selected in this study for bioethanol production, which highlights the originality of this study.

However, studies reporting phytopathogenic action of the fungi *E. endophytica* (Douanla-Meli & Scharnhorst, 2021), *N. cubana* (Li, Li, & Yang, 2021), and *F. pseudocircinatum* (Santillán-Mendoza et al., 2018; Kee, et al., 2020) were found. Furthermore, Ferreira et al. (2016) studied association of *E. endophytica* in medicinal plant from Amazon region with production of bioactive compounds against amastigotes forms of *Trypanosoma cruzi*.

3.2 Activity enzyme

The fungi *E. endophytica*, *N. cubana* and *F. pseudocircinatum* showed 1.40; 1.67 and 1.11 U/ mL as the best averages of amylolytic enzyme activity under the conditions of the present study, T= 50° C and pH= 4.8 (Ghose, 1987), respectively. However, Beltagy, Abouelwafa, & Barakat (2022), studying α-amylases purified from *Aspergillus flavus*, observed amylolytic activities at T= 50° C (71.5 U/mL) and pH= 6.0 (65.17 U/mL)

Silva Santiago et al. (2022) when studying enzymatic activity of CMCases found that *F. pseudocicinatum* showed peak enzymatic activity of 0.309 U/mL. However, Aguiar (2017) and Almeida (2015) analyzed the amylase activity from filamentous

fungi and they verified that the highest amylolytic activities were of *Aspergillus niger* (1.09 U/mL) and *Aspergillus brasiliensis* (3.8 U/mL), respectively.

The average amylolytic activity (U/mL), estimated total protein content (mg/mL), and average specific activity (U/mg) are described in Table 1.

Table 1. Enzyme activity, total protein content and specific activity of fungi.

Fungi	Activity (U/mL)	Proteins (mg/mL)	Specific activity (U/mg)
E. endophytica	1.40 ± 0.145	0.828	1.69 ± 0.175
N. cubana	1.67 ± 0.016	0.894	1.90 ± 0.018
F. pseudocircinatum	1.11 ± 0.037	0.865	1.28 ± 0.043

Source: Authors.

3.3 Temperature and pH

The influence of temperature and pH are determining factors in the enzymatic activity and, in effect, influence the release of glucose molecules useful for bioethanol production. Our study presented as optimal temperature (T) and pH points, respectively, T= 50° C (Figure 1) and pH= 5.0 (Figure 2).

We observed that at 60° C, the enzymatic activities remained between 48% and 60% of the maximum enzymatic activities and more than double the activity at the initial T (20° C). However, after 60° C there is a progressive loss of fungal enzymatic activities, *i.e.*, the losses of amylolytic activities (LAA), in relation to the activities at initial T, reach, 62.9%, 74.8%, and 51.8% at 90° C, as described in Table 2.

We further note, that small variations around the optimum pH value cause reduction or loss of amylolytic enzyme function (Figure. 2).

Table 2. Enzymatic activity (U/mL) as a function of temperature and loss of amylolytic activity.

Amylase/Fungi	20° C	30° C	40° C	50° C	60° C	70° C	80° C	90° C	LAA (%)
E. endophytica	0.332	0.385	0.578	1.500	0.724	0.466	0.280	0.123	62.9
N. cubana	0.354	0.592	0.706	1.603	0.975	0.620	0.252	0.089	74.8
F. pseudocircinatum	0.243	0.367	0.460	0.957	0.592	0.326	0.218	0.117	51.8

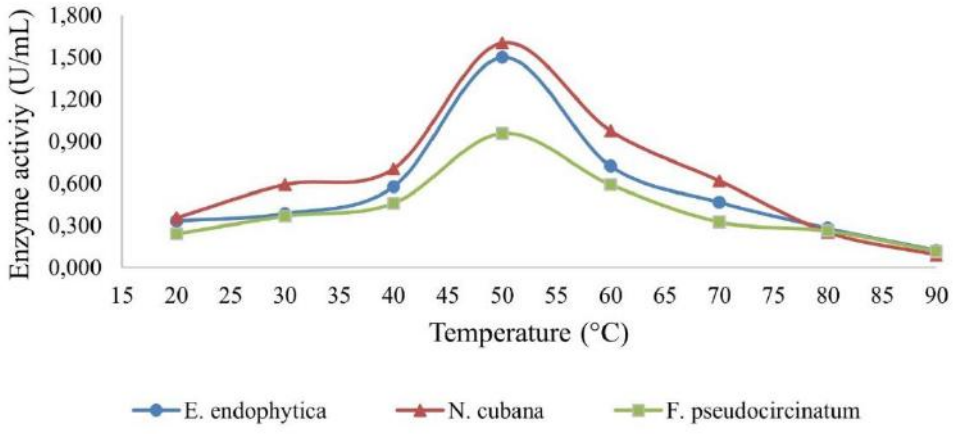
Note. LAA - relative loss of amylolytic activity by temperature variation 20 - 90° C.
Source: Authors.

Beltagy et al. (2022) showed that α-amylases expressed from *Aspergillus flavus* fungi had thermal stability at 50 °C where they still maintained about 96.1 % of the initial activity, and at temperatures of 60°C and 70 °C the α-amylases still maintained more than 51.2 % of the initial activity.

Silva et al. (2018) when analyzing the thermal stability and pH of enzymes from *Trichoderma reesei* observed that temperatures around 55 °C and pH= 2 - 11 showed higher enzyme activities, but at temperatures above 60 °C the enzyme activity is strongly reduced, indicating thermal degradation of the enzymes. Marco et al. (2015) studied the characterization of the thermostability of β-mannanase from *Aspergillus foetidus* and determined the optimal temperature and pH, respectively, 60° C and pH variable between 3.0 - 9.0.

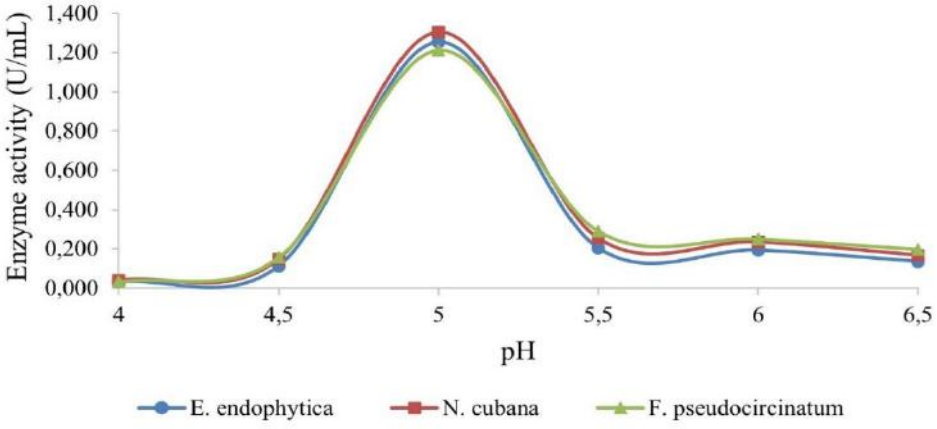
We found in our study that the combination of temperature 50 °C and pH = 5.0 showed the maximum for amylolytic enzyme activity and therefore favor the release of glucose in the bioreactor medium in SSF. Figures 1 and 2 show the behavior of the enzymatic activity of each fungus as a function of T and pH, respectively.

Figure 1. *O*ptimal temperature as a function of amylolytic activity.



Note. peak enzyme activity: *E. endophytica* = 1.5, *N. cubana* = 1.6, e *F pseudocircinatum* = 0.96.
Source: Authors.

Figure 2. Optimum pH as a function of amylolytic activity.

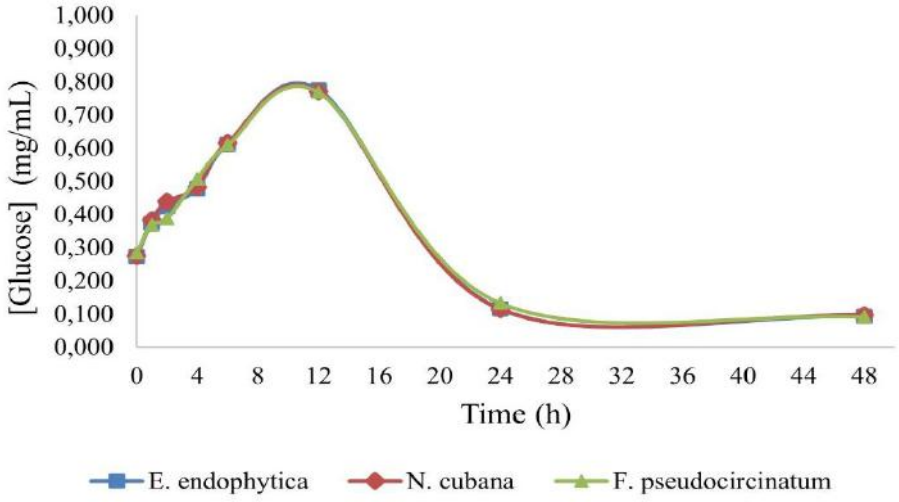


Note. peak enzyme activity: *E. endophytica* = 1.25, *N. cubana* = 1.3, e *F pseudocircinatum* = 1.21.
Source: Authors.

3.4 Bioethanol production

For SSF and thus bioethanol production, the temperature was kept at 30 °C for optimization of fermentation by *S. cerevisiae* as performed by Gronchi et al. (2019) and pH= 5.0 in the fermentation bioreactors. However, Zhang et al (2013) adjusted in the bioreactors pH= 4.0 and temperature at 30 °C for SSF assay for ethanol production from sweet potato root. Figure 3 shows the average determined concentration (mg/mL) of glucose throughout the SSF process.

Figure 3. Average concentration (mg/mL) of glucose released in SSF.



Note. [glucose] (12 h): *E. endophytica* = 0.768; *N. cubana* = 0.770; *F. pseudocircinatum* = 0.775.
Source: Authors.

As seenin Table 3, the bioethanol production yield of the fermented samples analyzed within 48 h bring the the samples analyzed in this study showing yields of 17.3 - 88.1 (%) of the expected theoretical yield (4.125 g/g glucose/L), with the sample (*F. pseudocircinatum*) having the highest recorded yield, *i.e.*, 3.635 g/g glucose/L.

Table 3. Average concentrations and yields in bioethanol production (48 h).

Sample/fungi	Concentration (% v/v)	Actual yield (%)	Actual yield (g/g glucose/L)
E. endophytica	0.077	17.3	0.715
N. cubana	0.140	31.5	1.300
F. pseudocircinatum	0.392	88.1	3.635

Source: Authors.

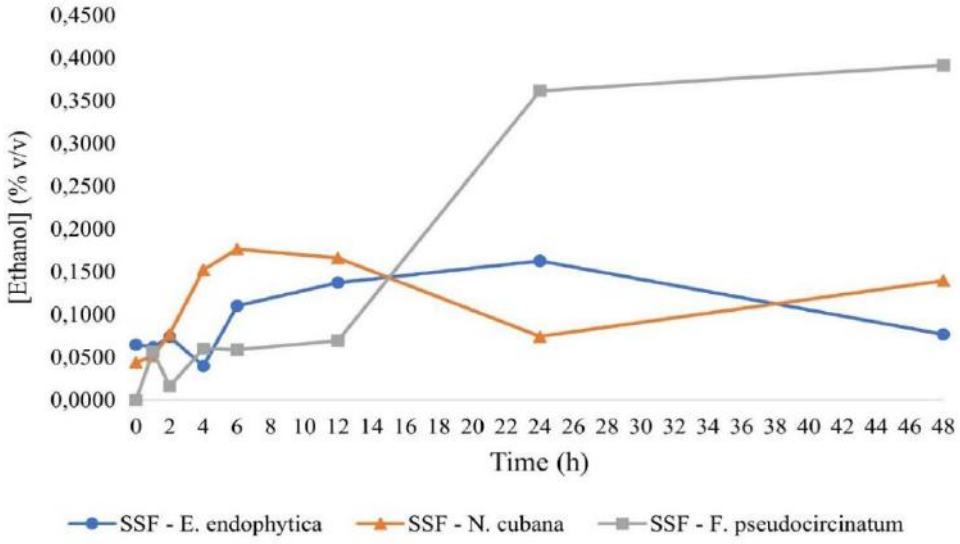
Beltagy et al. (2022) produced bioethanol from free amylases from *Aspergillus flavus* and *S. cerevisiae* incubated simultaneously, whose actual yield achieved was 0.07 g/ g sugar/L. However, amylases from *Fusarium oxyparum* were used on starch-rich foods for conversion into bioethanol with the help of *S. cerevisiae* and thus yield of 20.6 g/L performed in SSF (Prasoulas et al., 2020).

Furthermore, using factorial strategy to evaluate bioethanol production from *Solanum lycicarpum* fruit starch and *Saccharomyces bayanus* yeast, Morais et al. (2019) obtained the highest bioethanol production when 1 % glucose equivalent was fermented in 48 h. Under these conditions the production yield was 92.13 %.

Using optimized cultivation factors for *Candida albicans*, the conversion rate of sweet potato starch to bioethanol was 0.426 g/g, and of potato starch was 0.437 g/g (73 %) (Aruna et al., 2014). However, Zhang et al. (2013) produced ethanol from sweet potato starch incubated simultaneously with *A. niger* and *Zymomonas mobilis* yeast and then obtained from 14.4 g ethanol/100 g starch, *i.e.*, 87% of the theoretical yield.

The maximum ethanol concentrations determined from the samples analyzed during 48 h were: 0.163 (24 h; *E. endophytica*), 0.177 (6h; *N. cubana*), and 0.392 (48; *F. pseudocircinatum*) (% v/v), as presented in Figure 4.

Figure 4. Ethanol concentrations determined over 48 hours.



Note. [ethanol] (% v/v): *E. endophytica*; *N. cubana, e F pseudocircinatum* = 0.137, 0.166, e 0.069 (12h); 0.163, 0.74, e 0.362 (24 h), e 0.77, 0.140, e 0.392 (48 h).
Source: Authors.

The lower concentrations of ethanol up to 12h of the samples (SSF - *F. pseudocircinatum*) may be due to the possible interference of other proteins in the bioreactor medium that delayed the amylolytic action on starch and, therefore, the release of oligosaccharides and glucose; or by the inhibitory action of proteins on the fermentative metabolism of yeast *S. cerevisiae*. However, after overcoming these possible influences, an increasing increase in ethanol concentration was achieved up to 48 h.

The fungi *N. cubana* and *E. endophytica* showed similar amylolytic activities. However, it is verified that possible interferences previously commented, had little influence in the sample (SSF - *N. cubana*) until 6 h, because it may have occurred synergistic action of amylases that released glucose and then facilitated the fermentation by the yeast. Then, there is a reduction in ethanol contractions possibly due to the rapid degradation of amylases or other chemical compounds that hindered the conversion of glucose into ethanol by the yeast

The samples (SSF - *E. endophytica*), in turn, presented lower variations in ethanol concentration during 48 h, which possibly demonstrates a stable amylolytic enzymatic action, therefore maintaining the fermentative process during this period. There was no statistically significant difference in bioethanol concentrations of the fungi in 48 hours, by Tukey test at 95% confidence level.

4. Conclusion

The amylases from the fungi *Endomelanconiopsis endophytica, Neopestalotiopsis cubana* and *Fusarium pseudocircinatum* are moderately tolerant to temperature variations and poorly tolerant to pH variations. However, this research showed that fungal amylases applied simultaneously with industrial *S. cerevisiae* on sweet potato starch showed performance in SSF and thus are potentially useful for ethanol production, as good yields could be obtained.

Further studies should be performed to evaluate other conditions as well as starch-rich substrates. It is suggested to perform enzyme purification studies and associate fermentative yeasts as well as thermophilic microorganisms simultaneously, or studies of immobilization of different amylases applied to SSF and comparative analysis of costs and energy yields in different conditions of fermentative processes for bioethanol production.

Acknowledgements

We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the grant provid to support biotechnological research, and also Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the funding of maintenance costs of CRC. We are also grateful to Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG) and Fundação de Apoio Científico e Tecnológico do Tocantins (FAPTO) for the research support.

We are grateful to Instituto Federal Goiano, *campus* Rio Verde, Brasil, for the support and for providing the Agricultural Microbiology and Technological Chemistry Laboratories and the Multi-User Analysis Center to perform the necessary assays. We are also grateful to the Analytical Chemistry Laboratory of the Instituto Federal de São Paulo, *campus* Matão, for the support in the assay performed.

We thank the Environmental Microbiology and Biotechnology Laboratory of the Universidade Federal do Tocantins (UFT) and also the post-graduate program in Biotechnology and Biodiversity of the Rede BIONORTE for all the support for this work.

References

Aditiya, H. B., Mahlia, T. M. I., Chong,W. T., Nur, H., & Sebayang, A. H. (2016). Second generation bioethanol production: A critical review. *Renewable and Sustainable Energy Reviews*. 66, 631–653. https://doi.org/10.1016/j.rser.2016.07.015

Aguiar Neto Filho, M. (2017). *Bioprospecção de fungos amilolíticos para produção de etanol*. [Unpublished master's dissertation, Instituto Federal Goiano].

Aitken, A., & Learnmoth, M. P. (2002). Protein determination by UV absorption. In: Walker, J.M. (Ed.), *The protein protocols handbook* (2nd ed., pp. 3-6). Totowa: Human Presse Inc. https://doi.org/10.1385/1-59259-169-8:3

Almeida, P. Z. (2015). *Diversidade do potencial amilolítico em fungos filamentosos: purificação e caracterização de uma glucoamilase de Aspergillus brasiliensis*. [Master's dissertation, Universidade de São Paulo]. http:// www.teses.usp. https://doi:10.11606/D.59.2015.tde-15052015-085904

Aruna, A., Nagavalli, M., Girijashankar, V., Ponamgi, S. P. D., Swathisree, V., & Rao, L.V. (2014). Direct bioethanol production by amylolytic yeast *Candida albicans*. *Letters in Applied Microbiology*, 60, 229–236. https://doi.org/10.1111/lam.12348

Astolfi, A. L. (2019). *Sacarificação e fermentação simultânea de biomassa de alga e amido e uso de resíduo do processo de fermentação para obtenção de biopetídeo*. [Master's dissertation, Universidade de Passo Fundo]. http:// http://tede.upf.br/jspui/handle/tede/1796

Beltagy, E. A., Abouelwafa, A., & Barakat, K. M. (2022). Bioethanol production from immobilized amylase produced by marine *Aspergillus flavus* AUMC10636. *The Egyptian Journal of Aquatic Research*. https://doi.org/10.1016/j.ejar.2022.02.003

Douanla-Meli, C., & Scharnhorst, A. (2021). Palm Foliage as Pathways of Pathogenic Botryosphaeriaceae Fungi and Host of New *Lasiodiplodia* Species from Mexico. *Pathogens*, 10(10), 1297. https://doi.org/10.3390/pathogens10101297

Favaro, L., Viktor, M. J., Rose, S. H., Viljoen-Bloom, M., & Casella, S. (2015). Consolidated bioprocessing of starchy substrates into ethanol by industrial *Saccharomyces cerevisiae* strains secreting fungal amylases. *Biotechnology and bioengineering*, 112(9), 1751-1760. https://doi.org/10.1002/bit.25591

Ferreira, M. C., Vieira, M. L. A., Zani, C. L., Alves, T. M. A., Sales Junior, P. A., Murta, S. M., & Rosa, L. H. (2016). Molecular phylogeny, diversity, symbiosis and discover of bioactive compounds of endophytic fungi associated with the medicinal Amazonian plant *Carapa guianensis* Aublet (*Meliaceae*). *Biochemical Systematics and Ecology*, 59, 36-44. http://dx.doi.org/10.1016/j.bse.2014.12.017

Ghose, T. K. (1987). Measurement of Cellulase Activities. *Pure & Applied Chemistry*., 59(2), 257-268. https://doi.org/10.1351/pac198759020257

Gonçalves, D. B., Batista, A. F., Rodrigues, M. Q. R. B., Nogueira, K. M. V., & Santos, V. L. (2013). Ethanol production from macaúba (*Acrocomia aculeata*) presscake hemicellulosic hydrolysate by *Candida boidinii* UFMG14. *Bioresource. Technology*, 146, 261–266. https://doi.org/10.1016/j.biortech.2013.07.075

Gronchi, N., Favaro, L., Cagnin, L., Brojanigo, S., Pizzocchero, V., Basaglia, M., & Casella, S. (2019). Novel yeast strains for the efficient saccharification and fermentation of starchy by-products to bioethanol. *Energies*, 12(4), 714. https://doi.org/10.3390/en12040714

International Energy Agency. (2021). *World Energy Balances*: Overview. https://www.iea.org/reports/world-energy-balances-overview

Kee, Y. J., Zakaria, L., & Mohd, M. H. (2020). Morphology, phylogeny and pathogenicity of *Fusarium* species from *Sansevieria trifasciata* in Malaysia. *Plant Pathology*, 69, 442–454. https://doi.org/10.1111/ppa.13138

Kumar, S., Grewal, J., Sadaf, A., Hemamalini, R., & Khare, S. K. (2016). Halophiles as a source of polyextremophilic α-amylase for industrial applications. *AIMS Microbiology*, 2 (1), 1-26. https://doi.org/10.3934/microbiol.2016.1.1

Layne, E. (1957.). Spectrophotometric and turbidimetric methods for measuring proteins. *Methods in Enzymology*, 3, 447-455. https://doi.org/10.1016/S0076-6879(57)03413-8

Li, L., Yang, Q., & Li, H. (2021). Morphology, Phylogeny, and Pathogenicity of Pestalotioid Species on *Camellia oleifera* in China. *Journal of Fungi* (Basel, Switzerland), 7(12), 1080. https://doi.org/10.3390/jof7121080

Machado, C. M. M., & Abreu, F. R. (2006). Produção de álcool a partir de carboidratos. *Revista de Política Agrícola*, 15 (3), 64-78. https://www.alice.cnptia.embrapa.br/handle/doc/121716

Marco, J. da D. C. I. de., Souza Neto, G. P. de., Castro, C. F. de S., Michelin, M., Polizeli, M. L.T.M., & Filho, E. X. F. (2015). "Partial Purification and Characterization of a Thermostable β-Mannanase from *Aspergillus foetidus*" *Applied Sciences*. 5(4), 881-893. https://doi.org/10.3390/app5040881

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31, 426-428. https://doi.org/10.1021/ac60147a030

Ministério das Minas e Energia, Governo Federal do Brasil. Balanço Energético Nacional – Ano base 2020. (2021). https://www.epe.gov.br/pt/publicacoes-dados-abertos/publicacoes/balanco-energetico-nacional-2021.

Morais, R. R., Pascoal, A. M., Pereira-Júnior, M. A., Batista, K. A., Rodriguez, A. G., & Fernandes, K. F. (2019). Bioethanol production from *Solanum lycocarpum* starch: A sustainable non-food energy source for biofuels. *Renewable Energy*, 140, 361-366. https://doi.org/10.1016/j.renene.2019.02.056

Papanikolaou, S., & Aggelis, G. 2002. Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. *Bioresource Technology*., 82(1), p. 43–49. https://doi.org/10.1016/S0960-8524(01)00149-3

Prasoulas, G., Gentikis, A., Konti, A., Kalantzi, S., Kekos, D., & Mamma, D. (2020). Bioethanol production from food waste applying the multienzyme system produced on-site by *Fusarium oxysporum* F3 and mixed microbial cultures. *Fermentation*, 6 (2), 39. https://doi.org/10.3390/fermentation6020039

Romão, T. C., Menezes-Filho, A. C. P., Harakava, R., Castro, C. F. S., & Morais, P. B. (2024). Molecular and morphological diversity, qualitative chemical profile and antioxidant activity of filamentous fungi of the digestive tract of *Phylloicus* sp. (Trichoptera: Calamoceratidae). *Brazilian Journal of Biology*, 84, e259983. https://doi.org/10.1590/1519-6984.259983

Santillán-Mendoza, R., Fernández-Pavía, S. P., O'Donnell, K., Ploetz, R. C., Ortega-Arreola, R., Vázquez-Marrufo, G., Benítez-Malvido, J., Montero-Castro, J. C., Soto-Plancarte, A., & Rodríguez-Alvarado, G. (2018). A Novel Disease of Big-Leaf Mahogany Caused by Two *Fusarium* Species in Mexico. *Plant Disease*, 102(10), 1965–1972. https://doi.org/10.1094/PDIS-01-18-0060-RE

Silva, F. L. da., Oliveira Campos, A. de., Santos, D. A. dos., Oliveira Júnior, S. D. de., Araújo Padilha, C. E. de., Sousa Junior, F. C. de., Macedo, G. R. de., & Santos, E. S. dos. (2018). Pretreatments of Carnauba (*Copernicia prunifera*) straw residue for production of cellulolytic enzymes by *Trichorderma reesei* CCT-2768 by solid state fermentation. *Renewable Energy*, 116, 299-308. https://doi.org/10.1016/j.renene.2017.09.064

Schweinberger, C. M., Putti, T. R., Susin, G. B., Trierweiler, J. O., & Trierweiler, L. F. (2016). Ethanol production from sweet potato: The effect of ripening, comparison of two heating methods, and cost analysis. *The Canadian Journal of Chemical Engineering*, 94(4), 716-724. https://doi.org/10.1002/cjce.22441

Schweinberger, C. M. (2016). *Inovação e optimzação no processo de produção de etanol a partir de batata-doce*. [Doctoral thesis, Universidade Federal do Rio Grande do Sul]. https://www.lume.ufrgs.br/handle/10183/143930

Silva Santiago, S. R. S. da., Santiago, P. A. L., de Oliveira, M. R., Souza Rodrigues, R. de., Barbosa, A. N., Silva, G. F.da., & de Souza, A. Q. L. (2022). Evaluation of enzymatic production of hydrolases and oxyredutases by Fusarium pseudocircinatum and Corynespora torulosa isolated from caesarweed (Urena lobata L., 1753). *Research, Society and Development*, 11(2), e13211225325-e13211225325. https://doi.org/10.33448/rsd-v11i2.25325

Souza Filho, P. F. de., Ribeiro, V. T., Santos, E. S. dos., & de Macedo, G. R. (2016). Simultaneous saccharification and fermentation of cactus pear biomass—evaluation of using different pretreatments. *Industrial Crops and Products*, 89, 425-433. https://doi.org/10.1016/j.indcrop.2016.05.028

Zhang, P., Chen, C., Shen, Y., Ding, T., Ma, D., Hua, Z., & Sun, D. (2013). Starch saccharification and fermentation of uncooked sweet potato roots for fuel ethanol production. *Bioresource Technology*, 128, 835–838. https://doi.org/10.1016/j.biortech.2012.10.166

Zhang, L., Zhao, H., Gan, M., Jin, Y., Gao, X., Chen, Q., Guan, J., & Wang, Z. (2011). Application of simultaneous saccharification and fermentation (SSF) from viscosity reducing of raw sweet potato for bioethanol production at laboratory, pilot and industrial scales. *Bioresource. Technology*, 102(6), 4573–4579. https://doi.org/10.1016/j.biortech.2010.12.115