Characterization of amylase, lipase and xylanase produced by actinobacteria cultivated in licuri [*Syagrus coronata* - (Martius) Beccari] residues

Caracterização de amilase, lipase e xilanase produzida por actinobactérias cultivadas em resíduos de licuri [*Syagrus coronata* - (Martius) Beccari]

Milena Santos Aguiar[1]; Andrea Limoeiro Carvalho[2]; Elizama Aguiar-Oliveira[1,*]

[1]Universidade Estadual de Santa Cruz (UESC), Rodovia Jorge Amado, km 16, Salobrinho, Ilhéus, Bahia, Brasil
[2]Universidade Estadual de Feira de Santana (UEFS), Avenida Transnordestina, s/n, Novo Horizonte, Feira de Santana, Bahia, Brasil
*Autor correspondente. E-mail: eaoliveira@uesc.br

ABSTRACT

In order to seach for different microbial sources of enzymes, *Arthrobacter polychromogenes* CDPI-30 and *Streptomyces violaceoruber* CDPA-32 were individually cultivated in licuri [*Syagrus coronata* - (Martius) Beccari] residues (shells and pressed cake). It was possible to detect some enzymes but amylase, lipase and xylanase were selected for a basic characterization. The optimum conditions were observed at pH 6 and 7 and 40 – 70 °C for amylases, pH 4 – 6 and 50 – 90 °C for lipases and pH 8 and 10 and 20 – 50 °C for xylanases. The best stabilities, evaluated under different conditions were, in a way, a result from the optimal conditions. Additionally, substrate saturation was not observed for soluble starch and xylan until 15 mg/mL and *p*-nitrophenyl palmitate until 9.95 µmol/mL. These results indicate that both, actinobacteria and the licuri residues, are good choices to produce basic industrial enzymes.

Keywords: CMCase, enzymatic activation, kinetic parameters, linearization, pectinase.

INTRODUCTION

The solid state fermentation (SSF) of fungi has been vastly proposed in the literature, mostly due to the simplicity (for example, agitation is not necessary and the cellular biomass is retained in the solid fraction which facilitates the downstream process) and to shorter time of cultivation (when compared to bacteria which have more complex cultivation conditions). However, the cultivation of actinobacteria is also capable of producing equally efficient multi-enzymatic extracts for multiple purposes and a great variety of metabolites, for example: acids, amino acids, vitamins, pigments (COSTA-GUTIERREZ et al., 2021), antibiotics and anticâncer drugs (JOSE,

1

MAHARSHI & JHA, 2021) and enzymes, such as, β-galactosidase (XU et al., 2011), cellulases (JOHN J. et al., 2022), chitinases (WANG et al., 2023), proteases (BOUBEKRI et al., 2022), xylanases (SOUZA et al., 2022) and others. Actinobacteria are, more commonly, identified as soil bacteria (ARAÚJO et al., 2020) and have been proposed, for example, for soil biorremediation (APARICIO et al. 2018) but they also have been cultivated in different agro-industrial residues – sugarcane bagasse and soil (RAIMONDO et al., 2020), corn straw (ZHAO et al., 2017), wheat bran, rice husk, rice bran and bagassae (KHANDEPARKAR & BHOSLE, 2006), wheat straw (BERROCAL et al., 2000), shrimp shell (WANG et al., 2023) and others – in order to obtain different metabolites. It is important to mention that the number of published papers with actinobacteria is still smaller compared to other bacteria and fungi, but it has been improving with the knowledge that they can be valuable producers of metabolites besides some unique characteristics of biotechnological interest (JAIN et al., 2022). In this present work, two actinobacteria, previously isolated from a cave in the Northeast of Brazil, identified as: *Arthrobacter polychromogenes* CDPI-30 and *Streptomyces violaceoruber* CDPA-32 (BISPO, 2010) were studied for the production of amylase, lipase and xylanase. Recently, it was also reported their ability to produce lipase and pectinase by SSF with licuri residues and wheat bran as substrate (RODRIGUES et al., 2022).

Licuri [*Syagrus coronata* - (Martius) Beccari] is a palm tree native to the Caatinga biome located in the semiarid region of Brazil (SOUZA et al., 2020) and, in this work, two residues from the processing of its fruits to obtain oil (fruit shells and pressed cake) were used as substrate for solid state fermentation (SSF) of the two above mentioned actinobacteria. Licuri cake can contain, for example: 230 g/kg of protein, 174 g/kg of cellulose, 229 g/kg of hemicellulose, 58.5 % (w/w FAME) of polyunsaturated fatty acids and 65.1 g/kg of ashes (SILVA et al., 2022); considering the composition of the fruit shells it has been reported, for example: 30.0 g/kg of protein, 218.9 g/kg of cellulose, 327.6 g/kg of hemicellulose, 382.0 g/kg of lignin, 4.7 g/kg of lipids and 2.3 g/kg of ashes (MENEZES et al., 2016). These data confirms that the two residues are rich substrates for SSF. The proposal for the valorization of residues/waste into other products (such as enzymatic solutions) has scientific and industrial recognition of its importance and is in line with circular economy concept (LIU et al., 2021).

Therefore, this present work reports the obtainance of crude multi-enzymatic extracts (CME) containing activities of three enzymes of great industrial interest: amylases, used for example to obtain different types of starch for the food industry (TESTER, QI & KARKALAS, 2006); lipases, which can be applied, for example, in detergentes and soaps (BHARATHI & RAJALAKSHMI, 2009) and xylanases, applyied, for example, in the hydrolisys of lignocelulosic material for bioethanol production (JUTUTU & WU, 2012). A basic characterization of theses three enzymatic activities identified in the obtained CMEs – in relation to pH, temperature, kinetic parameters and stability – was also performed and it can contribute to the scarce data about these enzymes obtained from SSF with licuri residues and actinobacteria. In view of the diversity of enzymes produced by actinobacteria, it is important to highlight that they can also be a valid choice for SSF with agro-industrial residues along with fungi. Furthermore, CME can be an effective choice for enzyme application when it is not necessary to use pure solutions which requires extensive and expensive downstream steps.

MATERIAL AND METHODS
Actinobacteria

Arthrobacter polychromogenes CDPI-30 and *Streptomyces violaceoruber* CDPA-32 were previously isolated by Bispo (2010), from the soil of the "Poço Azul" cave located in the Chapada Diamantina (Bahia, Brazil) region – coordinates 12°46'55.7"S 41°08'56.7"W (Figure 1) – and originally belonging to the *Laboratório de Pesquisa em Microbiologia* (LAPEM) of the State University of Feira de Santana - UEFS (Feira de Santana, Bahia,

Brazil). The protocols for activation and inoculum preparation (YM medium, 28 °C / 12 days) were described in detail by Aguiar et al. (2022).

Figure 1. Location of "Poço Azul" (Chapada Diamantina, Bahia, Brazil) – coordinates 12°46'55.7"S 41°08'56.7"W. Source: Google Maps

Licuri residues

The residues of licuri (*Syagrus coronata* - (Martius) Beccari): shells (external part of the fruits which is removed to obtain the seeds) and pressed cake (obtained after pressing of the seeds to obtain vegetable oil), were purchased from the *Cooperativa de Produção da Região de Piemonte da Diamantina* (COOPES) (Capim Grosso, Bahia, Brazil). The procedures for pre-treatment of these residues (drying and oil removing with hexane) were described in detail elsewhere (AGUIAR et al., 2022).

Solid state fermentation and multi-enzymatic crude extract obtention

The protocol for solid state fermentation of *A. polychromogenes* CDPI-30 and *S. violaceoruber* CDPA-32 in 20 g of a mixture (substrate) 7:3 (w/w) of licuri cake and licuri shells was detailed described in Aguiar et al. (2022), humidity was set as 70 % (v/w) with water and nutrient solution. Incubation took place in a B.O.D. incubator (Eletrolab, EL101/1) at 28 °C / 12 days. After fermentation, the crude multi-enzymatic extracts (CME) of each strain were obtained by water extraction and centrifugation, as described previously (AGUIAR et al., 2022; RODRIGUES et al., 2022). The total protein content of each CME was determined by Bradford (1976) methodology with bovine albumin as a standard.

Determination of the enzymatic activities

The activities of amylase, CMCase, FPase, laccase, lipase, pectinase, tannase and xylanase were determined in triplicate by colorimetric methodologies and a spectrophotometer (2000UV, BEL) based on: Bonine (2001), Ghose and Bisaria (1987), Lu et al. (2013), Sharma et al. (2000), Umsza-Guez et al. (2011) and Vasconcelos et al. (2013). For xylanase, pectinase, CMCase, FPase and amylase, the activities were based on the release of reducing sugars (expressed as xylose, *D*-galacturonic acid and glucose) determined by the DNS (3,5-dinitrosalicyclic acid) methodology with the absorbance measured at 540 nm. For xylanase, the reaction occurred with 10 mg/mL of xylan from birchwood in sodium acetate buffer (50 mM / pH 5) at 50 °C / 10 min; for CMCase, the reaction occurred with CMC (carboxymethyl cellulose) in sodium citrate buffer (50 mM / pH 4.8) at 50 °C / 10 min; for FPase a piece of Whatman filter paper n° 1 measuring 0.5 cm x 3.0 cm in sodium citrate buffer (50 mM / pH 4.8) at 50 °C / 60 min was applied; for general amylases 10 mg/mL of soluble starch in potassium phosphate buffer (100 mM / pH 7.0) at 50 °C / 30 min were necessary, and, for pectinase, citric pectin in sodium

acetate buffer (200 mM / pH 5) at 60 °C / 10 min was employed. For laccase, ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] in sodium acetate buffer (0.05 M / pH 4) at 40 °C / 5 min was used and absorbance was measured at 420 nm. Tannase activity employed methyl gallate in sodium citrate buffer (50 mM / pH 5) at 33 °C / 5 min and the release of gallic acid was determined at 520 nm. For lipase, 7.95 μmol/mL of pNPP (4-nitrophenyl palmitate) in potassium phosphate buffer (50 mM / pH 7) was applied and the reaction occurred at 37 °C / 5 min, with the release of 4-nitrophenol measured at 410 nm. The enzymatic activities were expressed as unit of enzymatic activity per gram of solid substrate used in the fermentation (U/g).

Basic characterization of enzymes

The obtained CMEs were applied to the basic characterization of enzymes. Based on previous studies from literature regarding enzyme characterization (CASTILHA et al., 2017; SANJIVKUMAR et al., 2017; SANCHEZ et al., 2019), the activities of amylases, lipase and xylanase were selected to estimate the optimum pH and temperature, kinetic parameters and pH and temperature stability described as follows. The activities were expressed as relative activities: [(U/U_o)·100%] with U_o being equivalent to the highest activity value detected in the range evaluated. The activities initially detected in the CMEs (Tab. 1) were equivalent to the basic conditions of pH, T and substrate concentration.

To obtain different pH ranges, different buffer solutions were used: sodium citrate (50 mM) and sodium acetate (100 mM) at pH 3 – 6, sodium phosphate (50 and 100 mM) at pH 7 and 8 and glycine-NaOH (100 mM) at pH 10. To determine the optimal temperatures, the range of 20 to 100 °C was selected. The substrate concentrations varied from 1.0 to 15 mg/mL of soluble starch and xylan and from 3.95 to 9.95 μmol/mL of pNPP. Additionally, for the estimative of the kinetic constants, K_m and V_{max}, the enzymatic activities obtained (V, U/g) were plotted against the substrate concentrations (S, mg/mL or μmol/mL), considering the linearization of Lineweaver-Burk – $(1/v)$ x $(1/S)$ – with at least four or five points.

The stability of each enzyme at different temperatures (35 – 75 °C) and pHs (3.0 – 10.0), was evaluated by estimating the deactivation constant (k_d, 1/min) and the half-life ($t_{1/2}$, min). For each enzyme, in triplicate, 0.6 mL of CME were added in 2.4 mL of standard buffer and incubation occurred in different temperatures for 120 min in a thermostatic bath (CE-160 - CINELAB). Aliquots of 400 μL were collected every 20 min and kept refrigerated (4 – 5 °C) until the end of the incubation time to determine activity. Triplicates of each activity in the collected samples (U) presented deviations bellow 2,2 % of the mean values (data not presented). The residual activities [ln(U/U_o)], using the mean values of activities, were plotted against the incubation time (t, min), and the k_d value was estimated by the slope of the linear adjustment. The $t_{1/2}$ was calculated based on the adjusted linear regression when: $U/U_o = 0.5$ and expresses the time necessary under the evaluated conditions to reduce the activity to half of its initial value.

RESULTS
Enzymatic screening

The crude multi-enzymatic extracts (CMEs) obtained for the actinobacteria CDPI-30 and CDPA-32 were investigated for some enzymes of biotechnological importance as an initial screening step of the project (AGUIAR, 2020). According to the results (Table 1), the obtained CMEs presented a promising enzymatic profile for: amylase, CMCase, lipase, pectinase and xylanase. However, for FPase, laccase and tannase, the obtained values (< 1 U/g) were very close to the control conditions of the reactions, thus indicating that for these enzymes, it would be more appropriate to quantify their activities by another methodology of higher precision (*e.g.* chromatography). Based

only on the highest activity values obtained (Tab. 1), amylase, lipase and xylanase were selected for basic characterization as presented in the next topics.

Table 1. Enzymatic (U/g of dry substrate) profile of crude extracts from *A. polychromogenes* CDPI-30 and *S. violaceoruber* CDPA-32 cultivated in licuri residues.

Enzymes	CDPI-30 (U/g)	CDPA-32 (U/g)
Amylase	135.0 ± 0.6^a	125.6 ± 0.2^f
CMCase	8.8 ± 0.4^b	14.9 ± 0.1^g
FPase	< 1.0	< 1.0
Laccase	< 1.0	< 1.0
Lipase	100.03 ± 1.0^c	72.3 ± 0.3^h
Pectinase	27.0 ± 1.4^d	39.0 ± 0.3^i
Tannase	< 1.0	< 1.0
Xylanase	300.0 ± 0.5^e	322.8 ± 1.2^j

Total protein content: CDPI-30: 0.021 ± 0.01 mg/mL and CDPA-32: 0.026 ± 0.01 mg/mL. Average values (triplicate) are presented as: mean value ± standard deviation. Values followed by different superscript letters (columns and lines) are statistically according to the Tukey test (95 % of confidence).

Basic enzymatic characterization

Amlyase

Amylases from both strains, CDPI-30 and CDPA-32, showed optimum activities at pH 7 (Fig.2a) and, within the pH range of 3.0 – 6.0, the residual activities (U/U_o) were around 80 %; for pH 8, however, the profiles showed an abrupt decay. The temperature profiles obtained (Fig. 2b) were more differentiated between the strains, and for CDPI-30 the optimal activity was obtained at 50 °C and for CDPA-32 at 60 °C. It is also important to note that for CDPI-30, the residual activities were up to 80 % in the range of 40 – 100 °C, and with CDPA-32 this range was restricted to 50 – 80 °C.
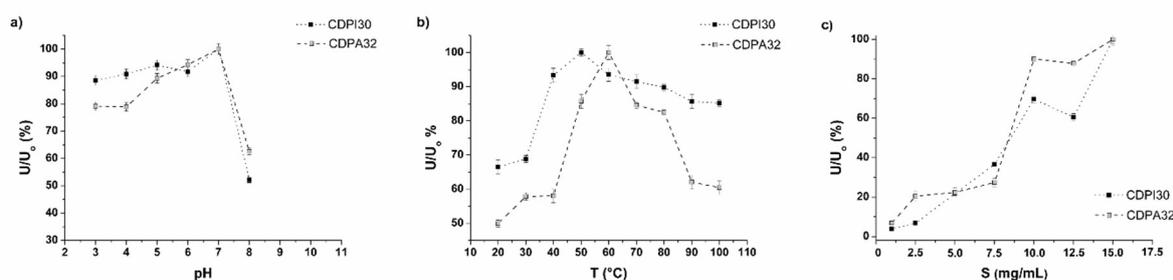

Figure 2. Characterization of amylase activity in crude multi-enzymatic extracts from *A. polychromogenes* CDPI-30 (black square) and *S. violaceoruber* CDPA-32 (gray square) in different **a)** pH, **b)** temperature and **c)** substrate (soluble starch) concentrations. Values were expressed as a residual activity (U/U_o, %), being 100 % equivalent to the highest observed activity value (U_o). Bars indicate the deviation of triplicates and lines are only used to guide the eyes. The standard conditions for activity were: pH 7.0, 50 °C and 10 mg/mL of starch.

Figure 3 presents the estimated values for the deactivation constant (k_d) and half-life ($t_{1/2}$). For both strains, at pH 4, the loss of activity showed a simple linear decay, but above pH 5.0, activation ($U/U_o > 100$ %) was

observed in the first 20 min, followed by a linear decay (Fig. 3.a and 3.c). Activation was maximal at pH 7.0 (optimum pH) with an increase in activities over the initial time of about 1.6 times for CDPI-30 and 2 times for CDPA-32. In addition, regarding $t_{1/2}$, pH 8 stood out for CDPI-30, but for both strains, pH 6.0 and 7.0 also resulted in $t_{1/2}$ above 300 min (Figs. 3.a and 3.c). Considering thermal stability, activation was also observed in the first 20 min of incubation (except at 30 °C) with activity values up to 30 % higher. For CDPI-30, the highest $t_{1/2}$ values (> 400 min) were estimated at 60 and 75 °C (Fig. 3.c) and for CDPA-32 at 45 and 75 °C (Fig. 3.d).

The substrate profiles of both strains were similar considering the evaluated range of starch concentration (Fig. 2.c), consequently, the estimated values for the kinetic parameters were also similar, as demonstrated in Table 2.
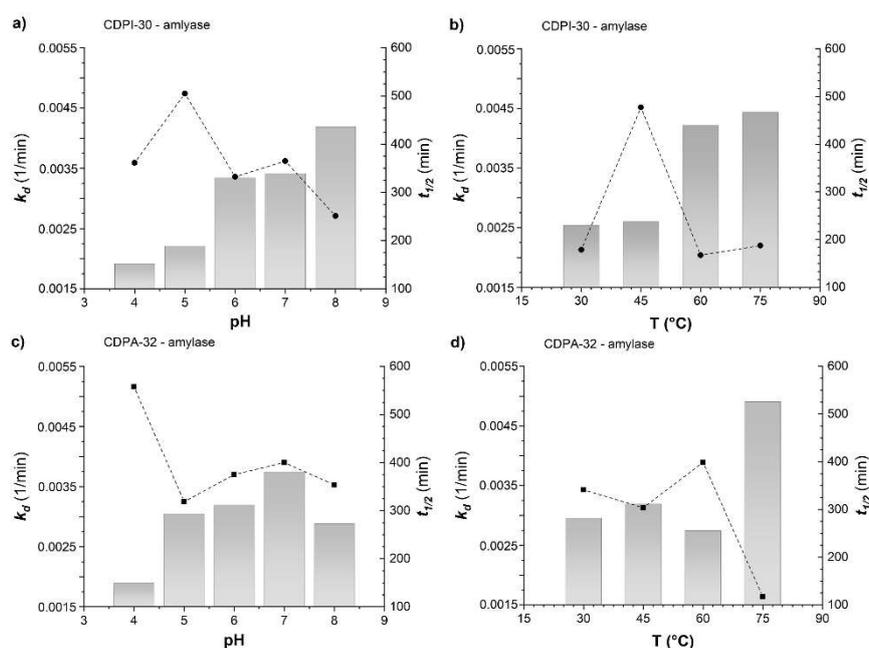
Figure 3. Estimative of **a)** and **c)** pH and **b)** and **d)** temperature (T) stability parameters: deactivation constant (k_d, 1/min) and half-life ($t_{1/2}$, min), for amylase in crude multi-enzymatic extracts from *A. polychromogenes* CDPI-30 and *S. violaceoruber* CDPA-32. Dotted lines are only used to guide the eyes. Linear Regression for k_d determination was obtained with four to five points and $0.9242 \leq R^2 \leq 0.9932$. The standard conditions for activity were: pH 7.0 and 50 °C.

Lipases

For lipase activity, pH 8.0 was identified as optimal for both strains, but residual activities between 80 to 90 % were obtained at pH 10.0 for both strains and at pH 7.0 for CDPI-30 (Fig. 4.a). Between 20 to 40 °C it was possible to observe the best activities ($U/U_o \geq 80$ %) for both strains, with optimum at 40 °C for CDPI-30 and 30 °C for CDPA-32 (Fig. 4b).

The substrate profiles for lipases were differentiated between the two strains analyzed (Fig. 4.c) and the estimated V_{max} for CDPI-30 was about five times higher than with CDPA-32, with similar K_m values (Tab. 2).

Table 2. Estimated kinetic constants: maximum velocity (V_{max}, 1/min) and Michaelis-Menten constant (K_m, g/L and μmol/mL) for the activities of amylase, lipase and xylanase from *A. polychromogenes* CDPI-30 and *S. violaceoruber* CDPA-32.

Amylase			
CDPI-30		CDPA-32	
V_{max} (1/min)	K_m (mg/mL)	V_{max} (1/min)	K_m (mg/L)
108.23	33.3	139.11	35.6
Lipase			
CDPI-30		CDPA-32	
V_{max} (1/min)	K_m (μmol/mL)	V_{max} (1/min)	K_m (μmol/mL)
66.18	17.42	13.97	11.77
Xylanase			
CDPI-30		CDPA-32	
V_{max} (1/min)	K_m (mg/mL)	V_{max} (1/min)	K_m (mg/mL)
191.84	32.5	82.81	20.5

* Linearization by Lineweaver-Burk plot with four to five points and $0.923 < R^2 < 0.999$.

Regarding the stability of lipases, at pHs 8.0 and 10.0 (Fig. 5.a and 5.c) – consistent with the optimal pH conditions – the best stabilities were observed and, consequently, higher $t_{1/2}$ values. However, as discussed before for amylases, only at pH 7.0, an effect of activation during the 120 min of incubation was observed with a linear increase up to 62 % for CDPI-30 and a smaller increase (47 %) for CDPA-32. For this reason, it was not possible to estimate the k_d values at pH 7.0. Activations were also observed at pH 8 only during the first 20 min of incubation reaching 97 % for CDPI-30 and 34 % for CDPA-32. Considering the temperature, the highest $t_{1/2}$ were estimated at 45 °C (Fig. 5.b and 5.d), a higher temperature than the optimum described above.
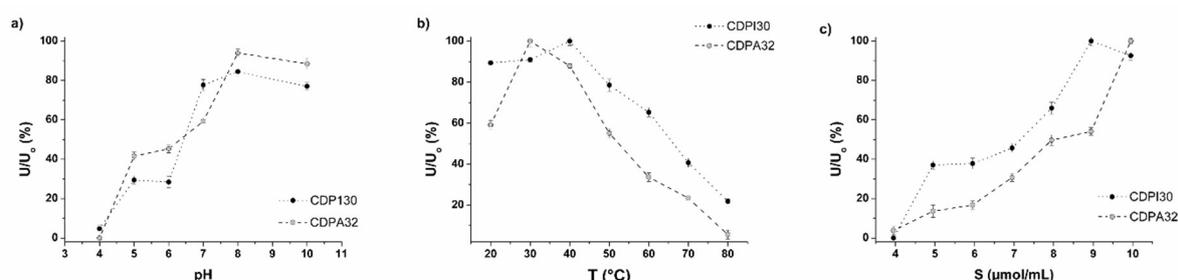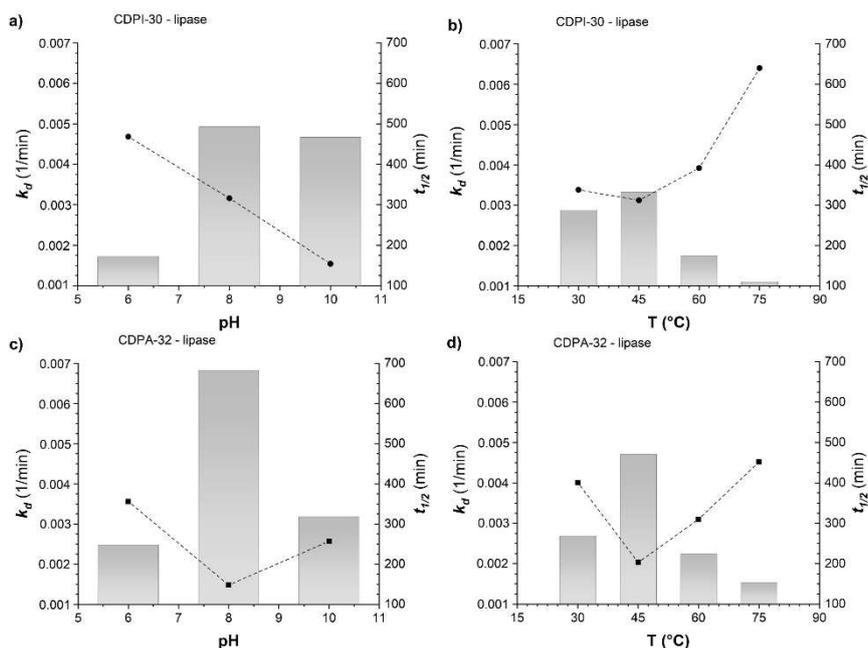

Figure 4. Characterization of lipase activity in crude multi-enzymatic extracts from *A. polychromogenes* CDPI-30 and *S. violaceoruber* CDPA-32 in different **a)** pH, **b)** temperature and **c)** substrate (*p*NPP) concentrations. Values were expressed as a residual activity (U/U_o, %), being 100 % equivalent to the highest observed activity value (U_o). Bars indicate the deviation of triplicates and dotted lines are only used to guide the eyes. The standard conditions for activity were: pH = 7.0, 37 °C and 7,95 μmol/mL of *p*NPP.
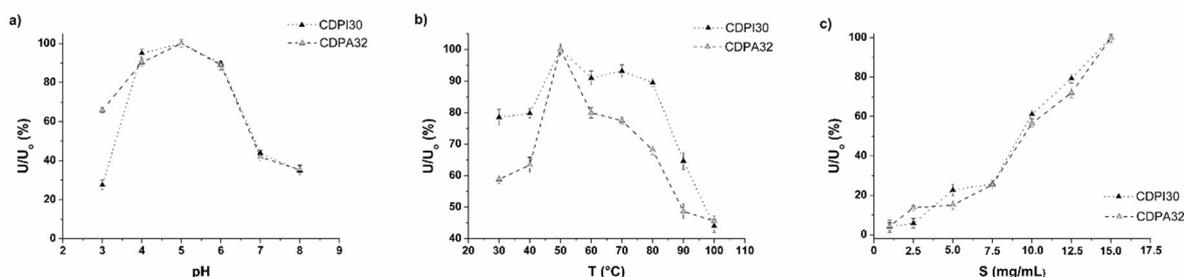
Xylanase

For the xylanase activities, the pH profiles (Fig. 6.a) were similar for both strains, with the pH range of 4.0 – 6.0 resulting in the highest residual activities ($U/U_o > 90$ %) with pH 5.0 as the optimal pH, while pH 3.0 was less favorable for CDPI-30 xylanases. Concerning the temperature (Fig. 6.b), at 50 °C the maximum activities

were observed, in addition, from 30 to 80 °C the relative activities were higher than 77 % for CDPI-30 and for CDPA-32 this range was reduced to 50 – 70 °C.

Figure 5. Estimate of **a)** and **c)** pH and **b)** and **d)** temperature stability parameters: deactivation constant (k_d, 1/min) and half-life ($t_{1/2}$, min), for lipases in crude multi-enzymatic extracts from *A. polychromogenes* CDPI- 30 and *S. violaceoruber* CDPA-32. Dotted lines are only used to guide the eyes. Linear Regression for k_d determination was obtained with four to six points and $0.9223 \leq R^2 \leq 0.9970$. The standard conditions for activity were: pH 7.0 and 37 °C.
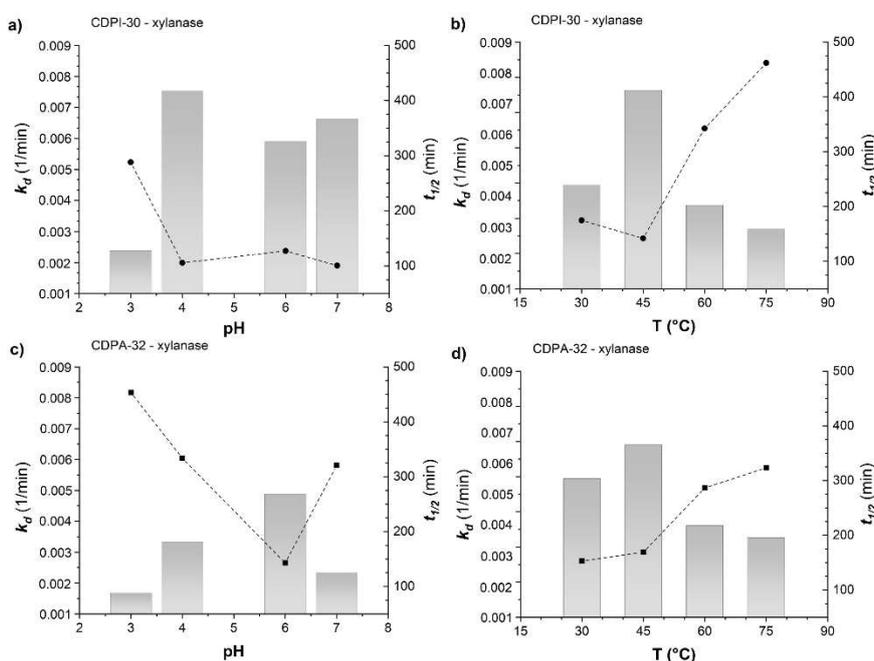

Figure 6. Characterization of xylanase activity in crude multi-enzymatic extracts from *A. polychromogenes* CDPI-30 and *S. violaceoruber* CDPA-32 in different **a)** pH, **b)** temperature and **c)** substrate (xylan) concentrations. Values were expressed as a residual activity (U/U_o, %), being 100 % equivalent to the highest observed activity value (U_o). Bars indicate the deviation of triplicates and the lines are only used to guide the eyes. The standard conditions for activity were: pH 5, 50 °C and 10 mg/mL of xylan.

The substrate profiles, within the analyzed range, were similar for both strains (Fig. 6.c) and the estimated K_m and V_{max} were, consequently, also similar (Tab. 3).

Regarding the pH stability (Fig. 7.a and 7.c), at pH 4, xylanase activities remained practically constant in the first 20 – 40 min of incubation followed by a linear decay. However, at pH 5 the stability profiles presented a crescent linear behavior over 120 min of incubation, with final residual activities increased by about 23 – 25 %; for this reason, it was not possible to determine k_d for pH 5; this also suggests enzymatic activation which can be

promoted at specific conditions. At temperatures of 45 and 60 °C (conditions around optimal temperatures), increases of 24 – 35 % in the residual xylanase activities were also observed in the first 20 min of incubation in both extracts.

Figure 7. Estimate of **a)** and **c)** pH and **b)** and **d)** temperature stability parameters: deactivation constant (k_d, 1/min) and half-life ($t_{1/2}$, min), for xylanases in crude multi-enzymatic extracts from *A. polychromogenes* CDPI- 30 and *S. violaceoruber* CDPA-32. Dotted lines are only used to guide the eyes. Linear Regression for k_d determination was obtained with four to six points and $0.9286 \leq R^2 \leq 0.9920$. The standard conditions for activity were: pH 5.0 and 50 °C.

DISCUSSION

Enzymatic screening

In comparison to fungi, actinobacteria may require a longer period of incubation. In this present work, for example, incubation occurred for 12 days, however, different incubation period have been reported such as 6 days (AL-DHABI et al., 2020) and 21 days (BERROCAL et al., 200). To overcome a longer (possible) incubation time, the application of crude multienzymatic extracts (CMEs) obtained from actinobacteria (instead of fermentation or the application of purified enzyme solutions) can present an interesting and economic alternative. For that reason, it is necessary to obtain and characterize different CMEs since, as a crude form, enzymes may exhibit more complex behaviors than purified forms due to, for example, the presence of isoforms with different behaviors as observed for amlyases (GEBREMARIAM et al., 2013) or the presence of compounds capable of interfering in their thermostability (SILVA et al., 2018). MECs may have sufficient enzymatic activities for certain processes that do not require enzymatic solutions with a high degree of purity and should be better explored in order to value enzymatic solutions as potent alternatives to fermentation processes. Still, for some specific applications, it is necessary to provide purified enzymatic solutions, as mentioned in the safety evaluation of a phospholipase, produced by a genetically modified *Streptomyces violaceoruber*, for food application (LAMBRÉ et al, 2023).

Actinobacteria have been suggested and investigated less than fungi for solid state fermentations (SSF), but they also have been identified as good sources of several enzymes, as confirmed by the results presented in Table 1, specially for CMCase, xylanase, amylase, pectinase and lipase. Another aspect worth mention is that

different actinobateria can produce the same enzyme but with different characterisitcs and it is crucial to perform characterization studies and analyze with caution when comparing results.

Cellulases (CMCase), exocellulases (FPase) and xylanases are important enzymes for the degradation of lignocellulosic material and, activities of 3.0 – 8.5 U/g of CMCase and 1.5 – 4.0 U/g of FPase, for example, have been reported in a study with four different actinobacteria (ZHAO et al., 2017). Another study reported activities around 400 to 5200 U/mL of xylanase by actinobacteria cultivated in synthetic medium and isolated from mangrove environment (SANJIVKUMAR et al., 2017). In addition, Sharma and Salwan (2018) suggested that actinobacterial xylanases may be more efficient than fungi xylanases due to the absence of co-secretion of cellulases. Laccase-like actinobacteria are also important because they participate in the specific degradation of lignin and *Streptomyces cyaneus*, for example, was able to reduce the lignin content of wheat straw by up to 19 % after 28 days (BERROCAL et al., 2000). Actinobacteria are also capable of degrading starch and pectic substances naturally found in different parts of plants. Amylase activity of 165.47 U/mL has been observed for *Streptomyces* sp. MBRC-82 (MANIVASAGAN et al., 2015) as well as a pectinase activity of 4857 U/g by *Streptomyces* sp. RCK-SC cultivated in supplemented wheat bran (ANISHA & PREMA, 2008). In relation to tannases, which are necessary for tannin degradation, one study found that among 22 tannase-positive bacteria isolated from olive mil waste (a residue rich in polyphenols), six of them were actinobacteria (FEDERICI et al., 2011). Additionally, among other strains, *Arthrobacter* was identified by Li et al. (2016) with the best potential for degradation of phenols. Considering lipases, although they were not identified for three strains of *Arthrobacter* by Pathma and Sakthivel (2013) but they were produced by *Actinomadura sediminis* UTMC 2870 (in a complex medium) reaching 300 – 1700 U/mL (IMANPARAST et al., 2018). Furthermore, five strains of *Streptomyce*s have also been reported as lipase producers (HAMEDI et al., 2019). All these exemples, also the results presented in this work (Tab. 1), can confirm that actinobacteria can be succesfully indicated as alternative sources (in relation to fungi) for industrial enzymes. To ilustrate more possibilities with both strains, CDPI-30 and CDPA-32, it has been recently reported CMEs containing, aproximatedly, 840 U/g and 15 U/g of lipases and pectinases, respectively, obtained from SSF with 67% (w/w) of humidity, 35 % (w/w) of licuri pressed cake, 35 % (w/w) of wheat bran and 30 % (w/w) of licuri shells under the same conditions of incubation as in this present work (RODRIGUES, et al., 2022).

Basic enzymatic characterization

Amlyase

In this present work (Tab. 2 and Fig. 1), amylases presented singular characteristics in comparison to other amylases from different actinobacteria, as observed, the optimum was restricted to pH 7.0 and not pH 8.0 as observed for other amylases. As an example, the α-amylase from *Streptomyces* sp. Al-Dhabi-46 presented optimal activities at pH 7.0 – 8.0 and 40 °C and, under these conditions, this enzyme was able to maintain up to 80 % of its activity for a shorter period (30 – 20 min) with lower $t_{1/2}$ values (50 – 55 min) (AL-DHABI et al., 2020). Another α-amylase from *Streptomyces* sp. D1 had the best activities in the pH range of 8.4 – 10.0 and demonstrated low resistance to incubation at temperatures above 80 °C, but with 6 h of incubation at 65 and 70 °C, it was possible to maintain 65 – 70 % of its initial activity (CHAKRABORTY et al., 2009). Longer periods of stability (to pH and temperature) were observe with amylases CDPA-32 and CDPI-30 (Tab. 2), for example, at 75 °C it was possible to estimate $t_{1/2}$ around 7.0 – 8.0 h and, at pH 7.0, $t_{1/2}$ was estimated around 5 – 6 h; pH 8.0 resulted in a higher value of $t_{1/2}$ (7 h) only for amylase CDPA-32.

Considering now the substrate for amylases in a CME from *Arthrobacter* sp. 226, the maximum activity was obtained with 10 mg/mL of starch but, with 15 mg/mL, 80 % of residual activity was still observed (OTTONI

et al., 2020); in this present work (Fig. 1c), it was not possible to observe a saturation point until 15 mg/mL. For a recombinant α-amylase from *Pseudoalteromonas* sp. 2-3, the kinetic constants were estimated as: V_{max} = 0.27 mg/(mL.min) and K_m = 6.94 mg/mL (SANCHEZ et al., 2019) which are lower values in comparison to what was calculated in this work (Tab. 3). It is also worth mentioning that in the two examples provided above, the conditions for determining amylase activity, especially temperature, were different from those applied for the amylases CDPA-32 and CDPI-30 since both actinobacteria exemplified are cold adapted. For that matter, it is necessary to investigate more amylases from actinobacteria so there can be more data available and, consequently, more possibilities for biotecnological application. Additionally, for the three selected enzymes in this presente work (amylase, lipases and xylanases), it is necessary to perform more detailed kinetic studies (with higher substrate concentration) so it is possible to know more about their biocatalitic performances.

The proposal of using crude multienzymatic extracts over purified enzymes has the advantage of reduced costs, but it is important to remember that the enzyme can present different properties in both forms. For example, the presence of isoforms with different behaviors can be observed with amylases (GEBREMARIAM et al., 2013), or maybe the presence of compounds capable of interfering in thermostability (SILVA et al., 2018). Amylases, for example, can bee applied to textile, paper or chemical industries without the necessity of purification as it is requested for food and drugs.

Lipase

As for to the optimum conditions observed for the lipases in this work (Fig. 4), a similar pH were observed with a lipase from *Janibacter* sp. R02 (around 7.0 and 8.0) (CASTILLA et al., 2017) and a partially purified lipase from *Streptomyces* sp. (pH = 7.0) (KUMAR et al., 2017). In regard to temperature, these two examples presented optimum at a higher temperature (80 °C) and a more similar conditions (40 °C), respectively. When considering pH 8.0, it was reported by Castilla et al. (2017) a reduction of around 20 % in thermophilic lipase activity after 1 h incubation in a wider temperature range (4 – 90 °C). More than 80 % of lipase activities, from *Streptomyces* sp. OC119-7, were preserved after a longer period of incubation (2 h) and wider pH and temperature ranges (5.0 – 11.0 and 20 – 60 °C) respectively (AYAZ et al., 2015). For lipases from *Arthrobacter* sp. incubated in pure vinyl acetate, stability was increased at 30 and 40 °C for 16 h, however, at 50 – 60 °C it was reduced to less than 50 % (YANG et al., 2009). The highest stability, expressed by $t_{1/2}$ values, were estimated in this present work (Tab. 4) at pH 8 for CDPI-30 lipase around 8 h and for CDPA-32 lipase around 11 h.

Activation was observed with the three inestigated enzymes (amylase, lipase and xylanase) during termal and pH stability determination. This effect has been reported for different enzymes, or maybe just ignore in a few cases since the activation can be quickly followed by the deactivation. Usually, with time, the enzyme activity tend to be lost due to unfolding of its 3D structure but, at certain temperature/pH/time, it is possible to observe an increase in activity (even for a brief period of time) and that is a complex behaviour that enzymes can present. According to Bechtold and Panke (2012), the enzymatic deactivation kinetics can be quite complex with a few intermediate protein states. In a complementary way, the activation caused by a brief incubation under certain temperature/pH can be applied before the enzyme application in order to improve its performance (AGUIAR-OLIVEIRA & MAUGERI, 2011). In order to better understand the activation/deactivation of amylase, lipase and xylanase from CDPI-30 and CDPA-32, it is necessary to perform more stability/thermodynamic studies. Even so, the results obtained so far are a good indicative of interesting properties of the crude extracts and for the purified enzymes.

When analalizing the V_{max} values (Tab. 3), for both lipases, it is possible to observe that lipase CDPA-32 could be more efficient (faster) than the lipase CDPI-30, considering the substrate *p*NPP. For the same substrate (*p*NPP), a purified lipase from *Acinetobacter* sp. AU07 (GURURAJ et al., 2015), presented a V_{max} closer to the

lipase from CDPA-32 (Tab. 3) but with a K_m up to 10 times smaller which could suggest a more effective kinetic (less substrate necessary to reach the same V_{max}). There are few reports in the literature on kinetic studies for actinobacterial lipases and the estimated kinetic parameters were obtained with different substrates, such as: $K_m =$ 873 mg/mL with 4-methylumbelliferyl butyrate (CASTILLA et al., 2017) and $K_m = 6.4$ mg/mL and $V_{max} = 21.0$ U/mg with pNP caproate (WANG et al., 2016).

In addition, besides the lipases produced by actinobacteria, interestingly, they also have the ability to grow using glycerol (COSTA-GUTIERREZ et al., 2021) – the principal waste generated from the esterification and transesterification of lipids (biodiesel production, for example) – this can perfectly picture the biotechnological value of actinobacteria.

Xylanase

By comparing the optimum conditions of pH = 5.0 (Fig. 6.a) and 50 °C (Fig. 6.b) observed with the xylanases from both strains with xylanases from other actinobacteria, it is possible to observe more basic values of pH (around pH 7.0 and 8.0) and a lower optimum temperature (40 °C) for the xylanase from *Streptomyces olivaceus* MSU3 (SANJIVKUMAR et al., 2017). For the xylanase from *Arthrobacter* sp., cultivated in residues of wheat, rice and bagasse, it was possible to observe a higher temperature range, 60 – 110 °C, and also a higher optimum pH, around 8 and 9 (KHANDEPARKAR & BHOSLE, 2006). In regard the stability of these two examples, the xylanase from *S. olivaceus* MSU3 presented better residual activities (reaching 77 – 79 %) when incubated at pH 8.0 / 40 °C / 90 min and the xylanase from *Arthrobacter* sp. was capable to retain 100 % of its activity at pHs 7.0 and 8.0 for 24 h. According to the results (Tab. 4) the best $t_{1/2}$ (around 7 h) were presented by the xylanase CDPA-30 in pH 4,0 and 45 °C, which are close to the optimum conditions. Morover, a few conditions evaluated (Tab. 4) presented periods of activation, similar fact was reported with the xylanase from *S. olivaceus* MSU3 which presented peaks of 15 – 20 % of activation in 30 and 90 min (SANJIVKUMAR et al., 2017).

Considering now the kinetics parameters (Tab. 3), between CDPI-30 and CDPA-32, the first one showed a V_{max} 2.3 times higher than the second one. Different parameters were reported in literature, for example, a purified xylanase from *S. olivaceus* MSU3: $V_{max} = 250.01 \pm 3.42$ mg/(min.mg) and $K_m = 8.16 \pm 2.17$ mg/mL with saturation above 20 mg/mL of xylan (SANJIVKUMAR et al., 2017) and a xlynase from *Arthrobacter* sp.: $V_{max} = 3571$ mg/(mg.min) and $K_m = 0.9$ mg/mL with saturation above 6 mg/mL of wheat bran (KHANDEPARKAR & BHOSLE, 2006).

With the intention to avoid expensive substrates (such as xylan), Danso and coworkers (2022) used wheat straw to cultivate *Streptomyces* sp. MS-S2 (isolated from the gut of termites) to obtain a crude extract containing xylanase and cellulase that was applied in the hydrolysis of the same agroindustrial residue to obtain reducing sugars for bioethanol production. This (and other similar works) is a good example to estimulate the studies and application of the strains CDPI-30 and CDPA-32 and their enzymes (crude or purified).

CONCLUSION

Actinobacteria are versatile for solid state fermentation (SSF), just as fungi can be, and their enzymes may present different characteristics than the "traditional" fungi enzymes, for this reason, it is important to promote more related studies with actinobacteria as a source for enzyme production through SSF. The results presented in this work, are good estimates for the behavior of amylases, lipases and xylanases from *A. polychromogenes* CDPI-30 and *S. violaceoruber* CDPA-32 and provided important information since there is a need for more current data on these enzymes from actinobacteria and SSF. As crude extracts, enzymes may exhibit more complex behaviors than purified forms, however, it is always worth noting that, in relation to operational costs, crude extracts with effective enzymatic activities obtained from microbial cultivation in agro-industrial residues are valuable

bioproducts. Basic characterization of enzymes in crude extracts can present some important information needed as an initial evaluation of the enzymes viabilities as crude extracts. The CMEs obtained resulted with good activities of amylases, lipases and xylanases (and indicated to contain more enzymes) and, concerning the application, these CMEs could be directed towards processes and formulations that do not require pure enzymes as usually required for food and drugs. In addition, licuri residues proved to be good substrates for SSF and the use of agro-industrial residues is a modern necessity since good results can be obtained from such underused materials. Sequential studies will give more detailed information so the biocatalytic performances can be better manipulated. To sum up, this work contributed with valid information of actinobacteria cultivated in agro-industrial residues as a source of industrial enzymes.

ACKNOWLEDGMENTS

REFERENCES

AGUIAR, M.S.; MALDONADO, R.R.; CARVALHO, A.L.; AGUIAR-OLIVEIRA, E. Characterization of enzymes produced by actinobacteria cultivated in licuri residues. In: DHARUMADURAI, D. (Ed.). **Methods in Actinobacteriology**. New York: Springer, p. 495, 2021. doi:10.1007/978-1-0716-1728-1_74

AGUIAR, M.S. *Caracterização das enzimas produzidas por actinobactérias cultivadas em resíduos de licuri*. Master Degree Dissertation. Ilhéus: Universidade Estadual de Santa Cruz (UESC), 2020, 94 p. (in Portuguese)

AGUIAR-OLIVEIRA, E.; MAUGERI, F. Thermal stability of the immobilized fructosyltransferase from *Rhodotorula* sp. **Brazilian Journal of Chemical Engineering**, v. 28, n. 03, p. 363-372, 2011. doi:10.1590/S0104-66322011000300002

AL-DHABI, N.A.; ESMAIL, G.A.; GHILAN, A.K.M., ARASU, M.V.; DURAIPANDIYAN, V.; PONMURUGAN, K. Isolation and purification of starch hydrolysing amylase from *Streptomyces* sp. Al-Dhabi-46 obtained from the Jazan region of Saudi Arabia with industrial applications. **Journal of King Saudi University - Sciences**, n. 32, v. 1, p. 1226-1232, 2019. doi:10.1016/j.jksus.2019.11.018

ANISHA, G.S.; PREMA, P. Cell immobilization technique for the enhanced production of α-galactosidase by *Streptomyces griseoloalbus*. **Bioresources Technology**, n. 99, v. 9, p. 3325-3330, 2008. doi:10.1016/j.biortech.2007.08.023

APARICIO, J.D.; RAIMONDO, E.E.; GIL, R.A.; BENIMELI, C.S.; POLTI, M.A. Actinobacteria consortium as an efficient biotechnological tool for mixed polluted soil reclamation: Experimental factorial design for bioremediation process optimization. **Journal of Hazardous Materials**, v. 342, p. 408-417, 2018. doi:10.1016/j.jhazmat.2017.08.041

ARAÚJO, R.A.; GUPTA, V.V.S.R.; REITH, F.; BISSETT, A.; MELE, P.; FRANCO, C.M.M. Biogeography and emerging significance of Actinobacteria in Australia and Northern Antarctica soils. **Soil Biology and Biochemistry**, v. 146, 107805, 2020. doi:10.1016/j.soilbio.2020.107805

AYAZ, B.; UGUR, A.; BORAN, R. Purification and characterization of organic solvent-tolerant lipase from *Streptomyces* sp. OC119-7 for biodiesel production. **Biocatalysis and Agricultural Biotechnology**, n. 4, v. 1, pp. 103-108, 2015. doi:10.1016/j.bcab.2014.11.007

BECHTOLD, M.; PANKE, S. Model-based characterization of operational stability of multimeric enzymes with complex deactivation behavior: An in-silico investigation. **Chemical Engineering Science**, n. 80, p. 435–450, 2012. doi:10.1016/j.ces.2012.05.030.

BHARATHI, D.; RAJALASHMI, G. Microbial lipases: An overview of screening, production and purification. **Biocatalysis and Agricultural Biotechnology**, n. 22, 101368, 2019. doi:10.1016/j.bcab.2019.101368

BISPO, A.S.R. ***Bioprospecção de actinomicetos isolados de solos no Estado da Bahia e seu potencial biotecnológico na produção de enzimas lignocelulolíticas***. Master Degree Dissertation. Cruz das Almas: Universidade Federal do Recôncavo da Bahia (UFRB), 2010, 93 p. (in Portuguese).

BERROCAL, M. et al. Biological upgrading of wheat straw through solid-state fermentation with *Streptomyces cyaneus*. **Applied Microbiology and Biotechnology**, n. 54, p. 764–771, 2000. doi:10.1007/s002530000454

BONINE, B.M. ***Produção de lipase pelo fungo <u>Mycelio phthora</u> sp. F. 2.1.4, caracterização e imobilização da solução enzimática bruta***. Master Degree Dissertation. São José do Rio Preto: Universidade Estadual Paulista (UNESP), 2011. 74 p. (in Portuguese).

BOUBEKRI, K.; SOUMARE, A.; MARDAD, I.; LYAMLOULI, K.; OUHDOUCH, Y.; HAFIDI, M.; KOUISNI, L. Multifunctional role of Actinobacteria in agricultural production sustainability: A review. **Microbiological Research**, v. 261, 127059, 2022. doi:10.1016/j.micres.2022.127059

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities utilizing the principle of protein-dye binding. **Analytical Biochemistry**, n. 72, v. 1-2, p. 248-254, 1976. doi:10.1016/0003-2697(76)90527-3

CASTILLA, A.; PANIZZA, P.; RODRÍGUEZ, D.; BONINO, L.; DÍAZ, P.; IRAZOQUI, G.; GIORDANO, S.R. A novel thermophilic and halophilic esterase from *Janibacter* sp. R02, the first member of a new lipase family (Family XVII). **Enzyme and Microbial Technology**, n. 98, p. 86-95, 2017. doi:10.1016/j.enzmictec.2016.12.010

CHAKRABORTY, S.; KHOPADE, A.; KOKARE, C.; MAHADIK, K.; CHOPADE, B. Isolation and characterization of novel α-amylase from marine *Streptomyces* sp. D1. **Journal of Molecular Catalalysis B – Enzymatic**, n. 58, n. 1–4, p. 17-23, 2009. doi:10.1016/j.molcatb.2008.10.011

COSTA-GUTIERREZ, S. B.; SAEZ, J. M.; APARICIO, J. D.; RAIMONDO, E. D.; BENIMELI, C. S.; POLTI, M. A. Glycerol as a substrate for actinobacteria of biotechnological interest: Advantages and perspectives in circular economy systems. **Chemosphere**, v. 279, 130505, 2021. doi:10.1016/j.chemosphere.2021.130505

DANSO, B.; ALI, S.S.; XI, R.; SUN, J. Valorisation of wheat straw and bioethanol production by a novel xylanase- and cellulase-producing Streptomyces strain isolated from the wood-feeding termite, *Microcerotermes* species. **Fuel**, v. 310, n. A, 122333, 2022. doi:10.1016/j.fuel.2021.122333

FEDERICI, E.; PEPI, M.; ESPOSITO, A.; SCARGETTA, S.; FIDATI, L.; GASPERINI, S.; ALTIERI, R. Two-phase olive mill waste composting: Community dynamics and functional role of the resident microbiota. **Bioresource Technology**, n. 102, v. 23, p. 10965-10972, 2011. doi:10.1016/j.biortech.2011.09.062

GEBREMARIAM, M.M.; ZARNKOW, M.; BECKER, T. Thermal stability of starch degrading enzymes of teff (*Eragrostis tef*) malt during isothermal mashing. **Process Biochemistry**, n. 48, v. 12, p. 1928-1932, 2013. doi:10.1016/j.procbio.2013.08.019

GHOSE, T.K.; BISARIA, V.S. Measurement of hemicellulase activities. Part 1: 472. Xylanases. **Pure and Applied Chemistry**, n. 591, p. 739-52, 1987. doi:10.1351/pac198759121739

GURURAJ, P.; RAMALINGAM, S.; DEVI, G.N.; GAUTAM, P. Process Optimization for Production and Purification of a Thermostable, Organic Solvent Tolerant Lipase From *Acinetobacter* sp. AU07. **Brazilian Journal of Microbiology**, n. 47, v. 3, p. 647-57. doi:10.1016/j.bjm.2015.04.002

HAMEDI, J.; KAFSHNOUCHI, M.; RANJBARAN, M. A Study on actinobacterial diversity of Hampoeil cave and screening of their biological activities. **Saudi Journal of Biological Sciences**, n. 26, v. 7, p. 1587-1595, 2019. doi:10.1016/j.sjbs.2018.10.010

IMANPARAST, S.; HAMEDI, J.; FARAMARZI, M.A. Enzymatic esterification of acylglycerols rich in omega-3 from flaxseed oil by an immobilized solvent-tolerant lipase from *Actinomadura sediminis* UTMC 2870 isolated from oil-contaminated soil. **Food Chemistry**, n. 245, p. 934-942, 2018. doi:10.1016/j.foodchem.2017.11.080

JAIN, S.; GUPTA, I.; WALIA, P.; SWAMI, S. Application of Actinobacteria in Agriculture, Nanotechnology, and Bioremediation. In: HOZZEIN, W.N. (Ed.). **Actinobacteria - Diversity, Applications and Medical Aspects** London: IntechOpen. 2022. doi:10.5772/intechopen.104385

JOHN J., A.; SAMUEL, M.S.; GOVARTHANAN, M.; SELVARAJAN, E. A comprehensive review on strategic study of cellulase producing marine actinobacteria for biofuel applications. **Environmental Research**, v. 214-3, 114018, 2022. doi:10.1016/j.envres.2022.114018

JOSE, P.A; MAHARSHI, A.; JHA, B. Actinobacteria in natural products research: Progress and prospects. **Microbiological Research**, v. 246, 126708, 2021. doi:10.1016/j.micres.2021.126708

JUTURU,1 V.; WU, J.C. Microbial xylanases: Engineering, production and industrial applications. **Biotechnology Advances**, n. 30, v. 6, p. 1219-1227, 2012. doi:10.1016/j.biotechadv.2011.11.006

KHANDEPARKAR, R.D.S.; BHOSLE, N.B. Isolation, purification and characterization of the xylanase produced by *Arthrobacter* sp. MTCC 5214 when grown in solid-state fermentation. **Enzyme and Microbial Technology**, n. 39, v. 4, p. 732-742, 2006. doi:10.1016/j.enzmictec.2005.12.008

KUMAR, P.P.; JANSI, R.S.; KUMAR, P.S.; CHRISTHUDAS, I.V.S.N.; RAJ, J.P.P.; VIJAYAKUMAR, A.; AGASTIAN, P.; IGNACIMUTHU, S. Optimization of biosynthesis parameters, partial purification and characterization of extracellular lipase from soil derived *Streptomyces* sp. Loyola Lipase-1. **Biocatalysis and Agricultural Biotechnology**, n. 17, p. 1878-8181, 2017. doi:10.1016/j.bcab.2017.10.011

LAMBRÉ, C.; BARAT BAVIERA, J.M.; BOLOGNESI, C.; COCCONCELLI, P.S.; CREBELLI, R.; CHESSON, A. Safety evaluation of the food enzyme phospholipase A2 from the genetically modified *Streptomyces violaceoruber* strain AS-10. **European Food Safety Authority Journal**, v. 21, I. 2, 7458, 2023. doi:10.2903/j.efsa.2023.7458

LI, F.; SONG, W.; WEI, J.; LIU, C.; YU, C. Comparative proteomic analysis of phenol degradation process by *Arthrobacter*. **International Biodeterioration & Biodegradation**, n. 110, p. 189-198, 2016. doi:10.1016/j.ibiod.2016.03.023

LIU, Y.; LYU, Y.; TIAN, J.; ZHAO, J.; YE, N.; ZHANG, Y.; CHEN, L. Review of waste biorefinery development towards a circular economy: From the perspective of a life cycle assessment. **Renewable and Sustainable Energy Reviews**, v. 139, 110716, 2021. doi:10.1016/j.rser.2021.110716

LU, L.; ZENZG, G.; FAN, C.; REN, X.; WANG, C.; ZHAO, Q. Characterization of a laccase like multicopper oxidase from newly isolated *Streptomyces* sp. C1 in agricultural waste compost and enzymatic decolorization of azo dyes. **Biochemical Engineering Journal**, v. 72, p. 70 –76, 2013. doi:10.1016/j. bej.2013.01.004

MANIVASAGAN, P.; VENKATESAN, J.; KANG, K.H.; SIVAKUMAR, S.; PARK, S.J.; KIM, S.K. Production of α-amylase for the biosynthesis of gold nanoparticles using *Streptomyces* sp. MBRC-82. **International Journal of Biological Macromolecules**, v. 72, p. 71-78, 2015. doi:10.1016/j.ijbiomac.2014.07.045

OTTONI, J.R.; SILVA, T.R.; PASSARINI, M.R.Z. Characterization of amylase produced by cold-adapted bacteria from Antarctic samples. **Biocatalysis and Agricultural Biotechnology**, v. 23, p. 101452, 2020. doi:10.1016/j.bcab.2019.101452

PATHMA, J.; SAKTHIVEL, N. Molecular and functional characterization of bacteria isolated from straw and goat manure based vermicompost. **Applied Soil Ecology**, v. 70, p. 33-47, 2013. doi:10.1016/j.apsoil.2013.03.011

RAIMONDO, E.E.; SAEZ, J.M.; APARICIO, J.D.; FUENTES, M.S.; BENIMELI, C.S. Coupling of bioaugmentation and biostimulation to improve lindane removal from different soil types. **Chemosphere**, v. 238, 124512, 2020. doi:10.1016/j.chemosphere.2019.124512

RODRIGUES, H.C.S.R.; CARVALHO, A.L.; SANTOS, L.M.; SILVA, A.B.; UMSZA-GUEZ, M.A. Actinobacteria hydrolase producer in solid-state fermentation using licuri. **Food Science and Technology**, v. 46, e020721, 2022. doi:10.1590/1413-7054202246020721

SANCHEZ, A.C.; RAVANAL, M.C.; ANDREWS, B.A.; ASENJO, J.A. Heterologous expression and biochemical characterization of a novel cold-active α-amylase from the Antarctic bacteria *Pseudoalteromonas* sp. 2-3. **Protein Expression and Purification**, v. 155, p. 78-85, 2019. doi:10.1016/j.pep.2018.11.009

SANJIVKUMAR, M.; SILAMBARASAN, T.; PALAVESAM, A.; IMMANUEL, G. Biosynthesis, purification and characterization of β-1,4-xylanase from a novel mangrove associated actinobacterium *Streptomyces olivaceus* (MSU3) and its applications. **Protein Expression and Purification**, v. 130, p. 1-12, 2017. doi:10.1016/j.pep.2016.09.017

SHARMA, S.; BHAT, K.T.; DAWARA, K.R. A spectrophometric method for assay of tannase using rhodanine. **Analytical Biochemistry**, v. 279, p. 85-89, 2000. doi:10.1006/abio.1999.4405

SHARMA, V.; SALWAN, R. Chapter 16: Biocontrol Potential and Applications of Actinobacteria in Agriculture. In: SINGH, B.P. et al. (Eds), **New and Future Developments in Microbial Biotechnology and Bioengineering**. New York: Springer, p. 93-108, 2018. doi:10.1016/B978-0-444-63994-3.00006-0

SILVA, L.F.; BARBOSA, A.M.; DA SILVA JÚNIOR, J.M.; OLIVEIRA, V.D.S.; GOUVÊIA, A.A.; SILVA, T.M.; OLIVEIRA, R.L. Growth, physicochemical properties, fatty acid composition and sensorial attributes from longissumus lumborum of young bulls fed diets with containing licuri cake: Meat quality of bulls fed licuri cake. **Livestock Science**, v. 255, 104775, 2022. doi:10.1016/j.livsci.2021.104775

SILVA, O.S.; OLIVEIRA, R.L.; SILVA, J.C.; CONVERTI, A.; PORTO, T.S. Thermodynamic investigation of an alkaline protease from *Aspergillus tamarii* URM4634: A comparative approach between crude extract and purified enzyme. **International Journal of Biological Macromolecules**, v. 109, p. 1039-1044, 2018. doi:10.1016/j.ijbiomac.2017.11.081

SOUZA, H.F.; BORGES, L.A.; GONÇALVES, V.D.D.P.; DOS SANTOS, J.V.; BESSA, M.S.; CAROSIA, M.F. KAMIMURA, E.S. Recent advances in the application of xylanases in the food industry and production by actinobacteria: A review. **Food Research International**, v. 162, n. B, 112103, 2022. doi:10.1016/j.foodres.2022.112103

SOUZA, J.V.; DE OLIVEIRA, A.P.D.; DA SILVA FERRARI, I.; MIYASATO, I.F.; DE FÁTIMA CARRIJO, K.; SCHWAN, R.F.; DIAS, F.S. Autochthonous and commercial cultures with functional properties in goat milk supplemented with licuri fruit. **Food Bioscience**, v. 35, p. 100585, 2020. doi:10.1016/j.fbio.2020.100585

TESTER, R.F.; QI, X.; KARKALAS, J. Hydrolysis of native starches with amylases. **Animal Feed Sciences and Technology**, v. 130, n. 1–2, p. 39-54, 2006. doi:10.1016/j.anifeedsci.2006.01.016.

UMSZA-GUEZ, M.A.; DÍAZ, A.B.; ORY, I.; BLANDINO, A.; GOMES, E.; CARO, I. Xylanase production by *Aspergillus awamori* under solid state fermentation conditions on tomato pomace. **Brazilian Journal of Microbiology**, v. 42, p. 1585-1597, 2011. doi:10.1590/S1517-83822011000400046.

VASCONCELOS, N.M.; PINTO, G.A.S.; ARAGÃO, F.A.S. *Determinação de Açúcares Redutores pelo Ácido 3,5-Dinitrosalicílico: Histórico do Desenvolvimento do Método e Estabelecimento de um Protocolo para o Laboratório de Bioprocessos*. **Boletim de Pesquisa e Desenvolvimento**. Fortaleza: EMBRAPA Agroindústria Tropical, n. 87, 24 p., 2013. Available at: https://www.embrapa.br/busca-de-publicacoes/-/publicacao/982130/determinacao-de-acucares-redutores-pelo-acido-35-dinitrosalicilico-historico-do-desenvolvimento-do-metodo-e-estabelecimento-de-um-protocolo-para-o-laboratorio-de-bioprocessos, last consult on March 10th, 2022. (in Portuguese)

WANG, J.L.; CHEN, Y.C.; DENG, J.J.; MO, Z.Q.; ZHANG, M.S.; YANG, Z. D.; LUO, X.C. Synergic chitin degradation by *Streptomyces* sp. SCUT-3 chitinases and their applications in chitinous waste recycling and pathogenic fungi biocontrol. **International Journal of Biological Macromolecules**, v. 225, p. 987-996, 2023. doi:10.1016/j.ijbiomac.2022.11.161

XU, K.; TANG, X.; GAI, Y.; MEHMOOD, M.A.; XIAO, X.; WANG, F. Molecular Characterization of Cold-Inducible β-Galactosidase from *Arthrobacter* sp. ON14 Isolated from Antarctica. **Journal of Microbiology and Biotechnology**, v. 21, n. 3, p. 236-242, 2011. doi:10.4014/jmb.1009.09010

YANG, G.; WU, J.; XU, G.; YANG, L. Improvement of catalytic properties of lipase from Arthrobacter sp.by encapsulation in hydrophobic sol–gel materials. **Bioresources and Technology,** v. 100, n. 19, p.4311-4316, 2009. doi:10.1016/j.biortech.2009.03.069

ZHAO, Y.; ZHAO, Y.; ZHANG, Z.; WEI, Y.; WANG, H.; LU, Q.; LI, Y.; WEI, Z. Effect of thermo-tolerant actinomycetes inoculation on cellulose degradation and the formation of humic substances during composting. **Waste Managment**, v. 68, p. 64-73, 2017. doi:10.1016/j.wasman.2017.06.022