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ENZYME CHARACTERIZATION OF ENDOPHYTIC ACTINOBACTERIA ISOLATED FROM TOMATO PLANTS

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ABSTRACT

Endophytic actinobacteria produce active metabolites, protecting plant tissues against stress. They compounds are widely used in human and veterinary pharmaceutics and agriculture, and of several enzymes with biotechnological potential. The present study characterizes the enzymatic production of endophytic actinobacteria colonizing tomato plants. Twenty-three actinobacteria isolates were tested for the detection of amylase, pectinase, cellulose, lipase, esterase, caseinase, gelatinase and catalase, at three temperatures (25°C, 28°C and 30°C) for 7 days. Substrate hydrolysis and enzymatic index (EI) were determined. The results showed that mean EI of all actinobacteria isolates was between 0.8 and 7.1. Although 30°C was the temperature at which the highest number of isolates presented enzymatic activity, it was at 25°C that amylase, pectinase, lipase and esterase activity reached the highest mean EI. Catalase was produced by all actinobacteria, while lipase, esterase and pectinase activity was observed for at least 20 isolates, regardless the incubation temperature. However, the highest number of isolates able to hydrolyze gelatin and cellulose (8) was observed when growth was carried out at 30°C. The highest EI means were observed for the hydrolysis of lipases, while the lowest values were seen for the hydrolysis of cellulose.

Keywords: Actinomycetes, enzymatic production, extracellular enzymes

1. INTRODUCTION

Actinobacteria are ubiquitous microorganisms widely distributed in natural ecosystems. The phylum comprises bacteria with high levels of guanine and cytosine in their DNA, whose morphology, physiology and relationship with oxygen follow considerably varied patterns [1]. These spore-forming Gram positive bacteria belong to the order Actinomycetes [2], and develop aerial mycelia, as well as in the substrate, when grown in solid media. They represent 20% to 60% of the total population of microorganisms living in the soil [3, 4]. Another typical characteristic of these microorganisms is the production of an odor similar to that of wet earth [5]. Actinomycetes are also observed in several other natural environments, such as fresh and salt waters, the rhizosphere, root nodules and inner plant tissues, decomposing organic matter, sediments, animal feces, activated sludge, and food products [6].

Some important characteristics of the genus *Streptomyces* include (i) the ability to colonize the rhizosphere, (ii) efficient biocontrol of phytopathogenic fungi, (iii) production of siderophores and growth-promoting substances *in vitro*, (iv) promotion of nodulation, and (v) an ancillary role in iron assimilation and nitrogen fixation by *Rhizobium* bacteroids in leguminous species [7, 8].

The biochemical heterogeneity, the ecological diversity and the exceptional capacity of these microorganisms to produce secondary metabolites make them an ideal target for the production of enzymes. Actinomycetes synthesize extracellular enzymes that hydrolyze complex macromolecules like proteins, starch, chitin, humus, cellulose and lignocellulose in different habitats. Furthermore, according to Pereira et al. [9], some enzymes produced by actinomycetes are also able to degrade substances known to be resistant to the attack by most bacteria and fungi, such as organic nitrogen compounds, steroids, aromatic compounds, acetylene, rubber and paraffins.

The several metabolic and physiologic properties of actinomycetes are behind their involvement in organic matter recycling processes and in the bioremediation of environments contaminated with xenobiotics and hydrocarbons. Additionally, these microorganisms take part in the conversion and production of biofuels [9] and act as important agents in the biocontrol of plant diseases [7] and this ability is mainly due to their capacity to interact with other microbial populations,

producing antibiotics and other secondary metabolites, with no damage to plants [10].

In this sense, based on the considerable economic and biotechnological importance that actinobacteria have as producers of large amounts of antibiotics and commercially interesting enzymes, the objective of the present study was to characterize the enzymatic production profile of endophytic actinobacteria isolated from tomato plants grown under different temperature conditions.

2. MATERIALS AND METHODS

2.1. Microorganisms

The endophytic actinobacterium isolates used in this work were obtained from tomato plant roots (*Lycopersicon esculentum*) collected in three cities in the state of Rio Grande do Sul, Brazil between 2005 and 2006 [8]. The isolates are deposited in the collection of microorganisms maintained by the Laboratory of Environmental Mycology, Department of Microbiology, Immunology and Parasitology, Basic Health Sciences Institute, Universidade Federal do Rio Grande do Sul, Brazil.

2.2. Enzymatic activity characterization

In total, 23 endophytic actinobacterium isolates were evaluated for their capacity to produce extracellular enzymes. Enzyme production, by the actinomycetes, was detected using solid culture media with a specific substrate for each enzyme (amylase, pectinase, cellulase, lipase, esterase, caseinase, gelatinase and catalase) produced. Gelatinase was detected using a semisolid medium. Actinobacteria cultures grown for 7 days in casein starch agar (2.0 g KNO_3, 2.0 g NaCl, 2.0 g K_2HPO_4, 0.05 g $MgSO_4$, 0.02 g $CaCO_3$, 0.01 g $FeSO_4$, 0.3 g casein, 10 g starch; 15 g agar; 1,000 mL distilled water) were inoculated using the spot method onto a Petri dish containing 20 mL of the specific culture media for each enzyme. After, dishes were placed in an incubator for seven days at 25°C, 28°C and 30°C in the dark. All assays were carried out in duplicate.

2.2.1. Amylase production

The consumption of starch by endophytic actinobacteria was assessed as described by [11]. Isolates were transferred to starch agar media containing 0.2% soluble starch. After incubation, 10 mL lugol solution (aqueous solution of iodine and potassium iodide) were poured onto colonies. Amylase production was detected as a transparent halo around the colony that typically signals starch hydrolysis.

2.2.2. Pectinase production

Pectinolytic activity was detected using tripticase soy agar (TSA) supplemented with 1% citric pectin. After incubation, 10 mL lugol solution was added to the colonies. Pectinase production was detected upon observation of a transparent halo caused by pectin hydrolysis, indicating positive pectinolytic activity.

2.2.3. Cellulase production

Cellulase production was assesses using a minimal mineral salt medium [12] supplemented with 0.5% carboxymethylcellulose (CMC) as the single carbon source. After incubation, cellulase production was detected adding a 0.1% solution of Congo red onto the colonies. After 15 min the solution was drained and media were washed with NaCl (1 M). After 30 min. the formation of an orange halo around the colony indicated cellulloytic activity.

2.2.4. Lipase and esterase production

The lipase and esterase activity of the isolates was evaluated according to the method described by Sierra [13] using culture media containing Tween 20 (polyoxyethylene sorbitan monolaurate) and Tween 80 (polyoxyethylene sorbitan monooleate), respectively. Culture media were sterilized and 1% (v/v) Tween 20 or Tween 80 previously sterilized by filtration was added. After incubation, dishes were stored at 4°C for 48 h to afford a better visualization of the halos. The production of lipase and esterase was confirmed upon observation of a whitish halo due to the formation of calcium crystals, contrasting with the transparent medium.

2.2.5. Protease production (caseinase and gelatinase)

The ability of actinomycetes to produce proteases was assessed using milk agar (solution A: 10.0 g low-fat powdered milk, 90 mL distilled water; solution B: 3.0 g agar, 97.0 mL distilled water) and gelatin agar medium (40.0 g gelatin, 3.0 g meat extract, 5.0 g peptone, 1,000 mL distilled water) to detect caseinase and gelatinase, respectively. After incubation, casein hydrolysis was observed as the presence of transparent zones surrounding the colonies. Gelatin hydrolysis was assessed in test tubes containing 4 mL specific medium and after incubation period the tubes were incubated under refrigeration at 4°C for 2 h upon confirmation of the liquid state of the medium.

2.2.6. Catalase production

Catalase production was assessed transferring actinobacterium isolates to nutrient yeast dextrose agar (NYDA) culture medium [14]. After a 7-day incubation period, 1 mL hydrogen peroxide 3% was added to colonies. Positive reaction was observed as the formation of bubbles upon addition of hydrogen peroxide [14].

2.3. Evaluation of enzymatic activity

Enzymatic activity was detected measuring hydrolysis halos and the colonies diameter on two directions. After, data were evaluated based on the determination of the enzymatic

index (EI) expressed as the ratio hydrolytic halo diameter: colony diameter [15]. For catalase and gelatinase activity, only the presence of enzymatic activity was assessed. Enzymatic indices were submitted to variance analysis and the Tukey's correlation test (α=0.05), when necessary, to compare means using the Statistica 8 software.

3. RESULTS

3.1. Enzymatic characterization

Extracellular enzyme production by endophytic actinobacterium isolates in solid media and specific substrates was observed based on the presence hydrolytic halos.

3.1.1. Amylolytic activity

Starch degradation in the culture medium was observed for 73.91% of isolates grown at 25°C and 28°C, and for 95.65% of isolates cultured at 30°C. Each isolate was able to degrade starch in at least one of the incubation temperatures determined in this work (Fig 1).

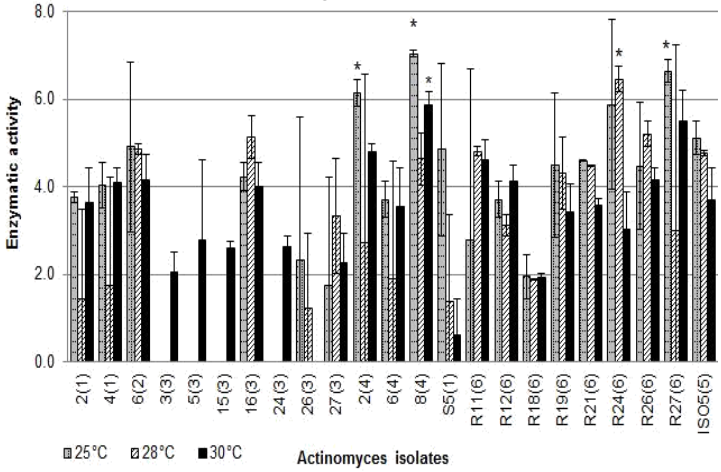

Fig 1. Amylolytic activity of actinomycete isolates assessed based on the degradation of soluble starch after incubation at 25°C, 28°C and 30°C for 7 days. (*) Different means according to the Tukey test (α=0.05).

Isolate 8(4) presented the highest amylolytic activity at 25°C and at 30°C, with EI of 7.04 and 5.86, respectively. High activity was observed for isolates 2(4) and R27(6), which showed statistically significant difference when grown at 25°C. For isolates incubated at 28°C, the highest starch hydrolysis index was observed for isolate R24(6) (EI=6.46), which differed statistically from the other isolates. On the other hand, 17.39% of isolates incubated at 25°C and at 28°C and 4.34% of those incubated at 30°C did not present degradation halo.

3.1.2. Pectinolytic activity

Most of the isolates show pectinolytic activity of these, isolates 4(1) and R12(6) presented EI of 14.0 and 9.0 when grown at 28°C and 25°C, respectively. The lowest pectinolytic indices were observed when isolates were incubated at 30°C. Isolates R11(6), R21(6) and R24(6) presented the highest pectinolytic indices at 30°C, which did not differ significantly (Fig 2). Also, 8.69% of isolates did not present pectinolytic activity at any temperatures used.

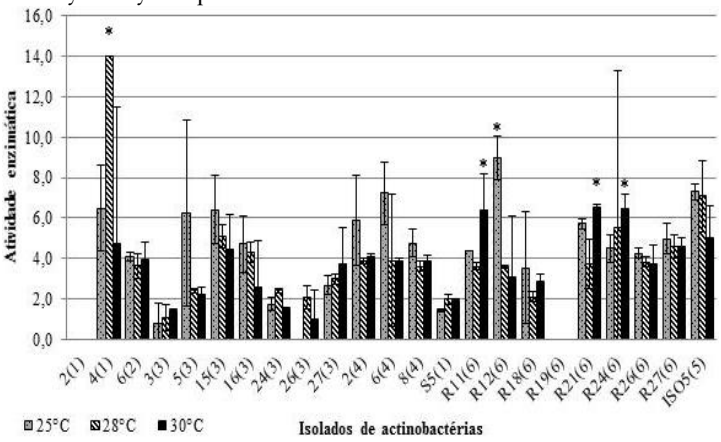

Fig 2. Pectinolytic activity of actinobacteria isolates assessed based on the consumption of citric pectin incubated at 25°C, 28°C and 30°C for 7 days. (*) Different means according to the Tukey test (α=0.05).

3.1.3. Lipolytic activity

Isolate R18(6) was the only isolate unable to degrade long-chain esters (Fig 3) at all temperatures assayed. On the other hand, the highest EI values were observed for isolate 8 R11(6) grown at 25°C and 30°C.

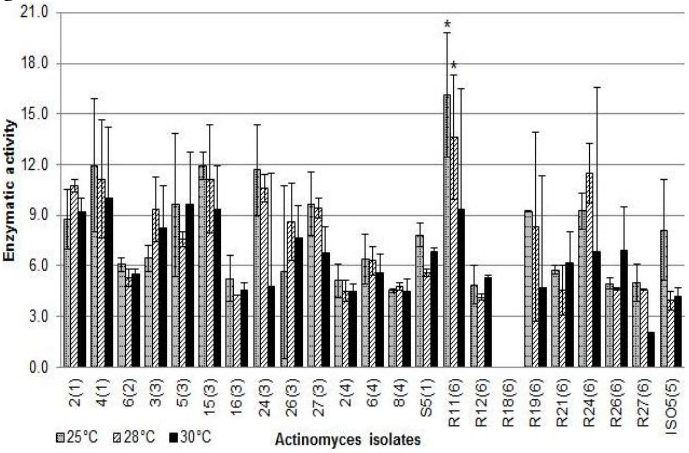

Fig 3. Lipolytic activity of actinobacterium isolates assessed based on the consumption of Tween 20 at 25°C, 28°C and 30°C for 7 days. (*) Different means according to the Tukey test (α=0.05).

3.1.4. Esterase activity

Enzymatic indices in the esters degradation assay were lower than those observed in the lipase degradation assay. However, 91.30% of isolates were able to degrade simple-chain esters at the three temperatures stipulated. The exception was isolate 3(3), which did not present detectable activity, and isolate 26(3), which only degraded esters at 30°C (Fig. 4). The highest esterase production values were observed

for isolates 4(1) (EI=6.8), 6(4) (EI=6.8) and R24(6) (EI=6.1) cultured at 25°C. In the assays carried out at 28°C and at 30°C the highest indices were 5.58 and 5.59 for isolates 15(3) and 4(1), respectively (Fig 4).

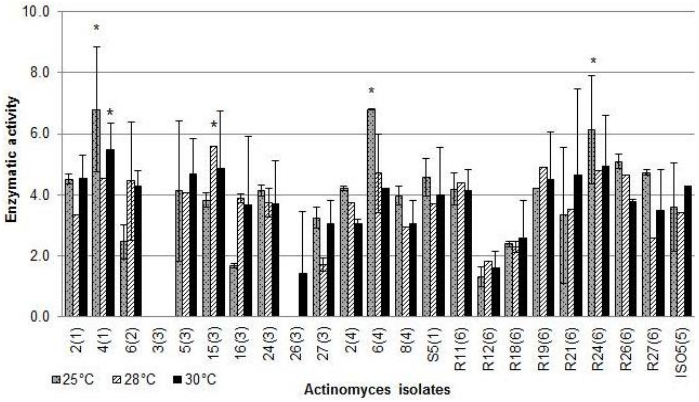

Fig 4. Activity of actinomycetes against esterase assessed based on the consumption of Tween 80 at 25°C, 28°C, and 30°C for 7 days. (*) Different means according to the Tukey test (α=0.05).

Table 1. Caseinase and cellulase activity of actinobacterium isolates assessed based on the degradation of milk casein and of carboxymethylcellulose, respectively, incubated at 25°C, 28°C and 30°C for 7 days.

Actinobacterium	Caseinase			Cellulase		
	25°C	28°C	30°C	25°C	28°C	30°C
2(1)	1.74	0.56	1.55	3.43	1.89	2.06
4(1)	0.88	0.00	0.00	3.05	3.75*	3.77*
6(2)	2.80*	3.04*	2.78*	0.00	0.00	0.00
3(3)	0.00	0.00	0.00	0.00	0.00	0.00
5(3)	0.00	0.00	0.71	0.00	0.00	0.00
15(3)	0.00	0.00	0.00	0.00	0.00	0.00
16(3)	1.35	1.73	1.94	0.00	0.00	0.00
24(3)	0.00	0.00	0.00	0.00	0.00	0.00
26(3)	0.00	0.00	0.00	0.00	0.00	0.00
27(3)	0.00	0.00	0.00	0.00	0.00	0.00
2(4)	1.35	1.62	1.75	0.00	0.00	0.00
6(4)	1.87	1.47	1.85	0.00	0.00	0.00
8(4)	1.38	1.51	1.82	0.00	0.00	0.00
S5(1)	0.00	0.00	0.00	0.00	0.00	0.00
R11(6)	1.38	1.35	1.41	0.00	0.00	0.00
R12(6)	0.00	0.00	0.00	1.64	2.33	2.37
R18(6)	0.00	0.00	0.00	2.70	2.55	3.30*
R19(6)	0.00	2.07	0.00	3.70	3.84*	4.18*
R21(6)	1.85	1.58	1.48	0.00	0.00	0.00
R24(6)	0.00	2.03	0.00	4.08*	4.04*	4.14*
R26(6)	1.92	1.53	1.87	3.20	0.00	0.63
R27(6)	1.66	1.87	1.74	0.00	0.00	0.00
ISO5(5)	2.26	1.89	2.05	0.00	1.67	0.89

(*) Different means according to the Tukey test (α=0.05).

3.1.5. Caseinase activity

Caseinase was detected based on the presence of hydrolysis halos in media containing casein as substrate. In total, 12 isolates (52.17%) degraded this substrate, considering all incubation temperatures. However, 11 isolates (47.82%) no presented caseinase activity at temperature tested. Therefore, the presence of caseinase was confirmed for endophytic actinobacteria in tomato plants, with isolate 6(2) presenting the highest EI (≥2.8) at the three temperatures stipulated (Table 1)

3.1.6. Cellulase activity

Of the 23 isolates tested, 30% of the isolates grown at 25°C and at 28°C were able to degrade the CMC present in the culture medium. Of the isolates cultured at 30°C, 34,78% exhibited this characteristic. Isolate ISO5(5) presented activity when incubated at 28°C and 30°C, while isolate R26(6) presented activity when incubated at 25 and 30°C (Table 1).

Isolate R24(6) presented EI≥4, the highest observed, independently of the incubation temperature. However, this index did not differ significantly from the index presented by isolates 4(1) and R19(6) incubated at 28°C and 30°C, and from that one obtained for isolate R18(6) at 30°C.

3.1.7. Gelatinase and catalase activity

Gelatinase activity was observed for an increasing number of isolates with increasing incubation temperatures (from 25°C to 30°C). The enzyme was produced by 8.69% (isolates 15(3) and 26(3)), 26.08% (isolates 5(3), 15(3), S5 (1), R12(6), R21(6), R27(6)) and 34.78% (isolates 3(3), 15(3), 27(3), 6(4), 8(4), S5(1), R12(6), R26(6)) of isolates grown at 25°C, 28°C and 30°C, respectively. For isolate 15(3), the production of this enzyme was very efficient, being able to degrade gelatin at all temperatures stipulated. A distinct behavior was observed for catalase, for which all isolates (100%) reacted with hydrogen peroxide.

4. DISCUSSION

Actinomycetes are characterized by the ability to produce a large variety of secondary, 12 biologically active metabolites, such as vitamins, enzymes and antibiotics [6]. These microorganisms or their enzymes have an array of biological industrial and environmental applications, like polymer hydrolysis, synthesis of chemicals, soil decontamination, biological control of diseases, and decomposition of organic matter.

The actinomycete isolates analyzed in the present study exhibited mean EI between 0.8 and 7.1, considering the three temperatures stipulated and the enzymes investigated. According to Fungaro and Maccheroni [16], EI values above 1.0 indicate enzymes with biotechnological potential. The degradation of specific substrates by actinobacteria presented

higher mean EI values when incubation was carried out at 25°C, while the highest number of isolates presenting activity was observed when incubation was conducted at 30°C. This may be due to the different nutritional and environmental demands of each isolate has concerning the optimal conditions to produce a given enzyme or other secondary metabolites. Variation in the production of enzymes by actinobacteria was reported by Duarte [17]. The study, with actinobacteria from soil, showed that changes in the incubation temperatures interfere with the capacity to produce enzymes, which is specific in terms of enzymatic activity. The author also observed that, except for gelatinase and lipase, the enzymatic activity amylase, caseinase, pectinase and cellulose were detected in a wide range of temperatures (25°C to 40°C). The results of the present study confirm this variation, showing that the degradation of different specific substrates is associated with incubation conditions. In this sense, the mesophile or thermophile character and the niche of isolates has to be considered in this kind of investigation. According to Gomes et al. [18], there is a narrow relationship between the niche taken by a microorganism and its intra- and extracellular enzymatic profile.

The degradation of starch, the most important organic compound in terms of energy storage, formed by two glucose polymers (amylose and amylopectin), was observed for 100% of isolates grown at three temperatures in the present study (Fig 1). The occurrence of amylase in actinomycetes is commonly observed in *Nocardia* and *Streptomyces* [19]. Our results are similar to the findings by Karanja et al. [20] who observed the amylolytic degradation with EI between 3.4 and 5.2 for all *Streptomyces* species isolated from soils in Kenya. Ayhsa et al. [21] reported an EI of 1.2 for *Actinomyces pyogenes*. The hydrolysis of this substrate by actinobacteria was also reported by Rodrigues [22], who analyzed the production of extracellular enzymes by 188 actinomycete isolates, observing a predominance of the genera *Nocardia*, *Nocardiopsis*, *Streptomyces* and *Terrabacter* in the degradation of starch. Sousa et al. [23], in a study on the role of *Streptomyces* isolates in the growth promotion of plants and in the biological control of phytopathogens, observed that the isolates investigated produced amylase, lipase and catalase.

Pectinases are present in plants and microorganisms, such as bacteria, yeasts and filamentous fungi [24]. These enzymes are used mainly in the juice, food [25] and paper [26, 27] industries. These enzymes are important for plants, since they play an important role in cell wall extension [28], infections [29], apart from helping in the maintenance of ecological balance due to their ability to decompose and recycle plant waste.

In the present study, 90.30% of isolates hydrolyzed citric pectin, with the highest EI observed for actinomycetes grown at 25°C (Fig. 2). Duarte [17] observed similar results in a study that evaluated the hydrolysis of citric pectin by actinobacteria isolated from soil, with a direct relationship between the rise in temperature from 25°C to 30°C and the drop in the number of isolates that produced the enzyme. In a study that characterized the production of pectinases by *Penicillium oxalicum*, Santi [30] observed that the optimal temperature for the production of polygalacturonase was 32ºC, while for pectin lyase and pectinesterase optimal values were observed at 28°C. According to Gummandi and Panda [31] the presence of several pectins in plant cells requires microorganisms to produce pectinases with different mechanisms of action to degrade these compounds.

Considering that endophytic bacteria used in the present study are mesophiles and that the pectin lyase degrades specifically the citric pectin, it is possible to suggest that the lyase in these isolates acts in the production of this enzyme at an optimal temperature of 25°C.

Cellulose is the most abundant polysaccharide in the plant biomass, accounting for between 20% and 50%, and is degraded by several enzymes, produced by a number of microorganisms, such as cellulases [32, 33]. These microorganisms play an important role in the decomposition of organic matter, mineralization of nutrients and in the promotion of plant growth. They also may act as agents in the biocontrol of some phytopathogenic fungi, like *Phytophtora* and *Pytium*, whose cellulose content in cell wall is between 17% and 35% [34]. Here, 26.08% of endophytic actinobacteria collected from tomato plants produced cellulases, independently of incubation temperature (Table 1). It is known that actinobacterium isolates from compost piles produced cellulases when incubated at temperatures between 35ºC and 37ºC [35, 22]. Similar results were obtained by Sousa et al. [23] in studies that observed a high number of actinobacteria (over 95%) able to produce cellulases by degradation of microcrystalline cellulose in the medium, when incubation temperature was 28±2ºC. Therefore, the detection of a low number of endophytic actinomycetes (8) isolated from tomato plants that were able to degrade cellulose in the present study may be linked to the use of CMC, a specific substrate, and to incubation temperatures (25°C, 28°C, and 30°C). It is possible to speculate that these isolates may show a better result for CMC hydrolysis when incubated at higher temperatures, since the number of isolates producing cellulase increased with temperature (Table 1) or more that CMC was not a good substrate for these isolates, some other assays must be done using different substrates in order to confirm this behavior of the isolates. Schrempf and Walter [36] observed that *Streptomyces reticuli* produces cellulases only when grown in microcrystalline cellulose, and that growth in glucose, glycerol or CMC inhibits the production of these enzymes, which present optimal activity at 55ºC and neutral pH. Li et al. [37, 38] reported the production of cellulases by *Streptomyces* spp. that present excellent activity at the 50ºC-55ºC temperature range. *Streptomyces rochei* produces endoglucanases and β-

glycosidase when grown at 42°C [39].

Of the *Streptomyces* species isolated by Ramirez and Coha [40], 92% presented cellulolytic activity in CMC in filter paper. Wirth et al. [41] analyzed 39 *Streptomyces* isolates, 11 degraded pure, amorphous or crystalline cellulose, 11 degraded colloidal forms but did not hydrolyze native and crystalline cellulose, and only 17 isolates were able to hydrolyze CMC. Similar results were observed by Wachinger et al. [42] in a study that analyzed 160 isolates: 100% hydrolyzed soluble cellulose, but only 15% hydrolyzed microcrystalline cellulose.

In the present study, of the 23 tested isolates to detect the production of lipases and esterases, over 91% were able to degrade long-chain and simple-chain esters at a 25°C-30°C temperature range. These results confirm the findings by Rodrigues [22] in a study that investigated actinobacteria from a compost pile. Lipases (olive oil) and esterases (Tween 80) were produced by 44% and 88% of isolates, respectively. In another study on soils impacted with oil-derived hydrocarbons but without history of contamination with hydrocarbons, the production of lipase and esterase by actinomycetes isolated from the soil was higher than 80%, while the actinomycetes isolated from contaminated soils presented degradation rate 10% higher from the results obtained with the actinomycetes from the non contaminated soil [17].

The biotechnological potential of *Streptomyces* to produce lipases and esterases was also reported by Karanja et al. [20]. The authors reported maximum EI of 4.2 and of 5.3for the degradation of Tween 20 and Tween 80, respectively. Here, the results obtained are more promising, with mean EI for lipases and esterases of 7.6 and 3.7, respectively, while for Tween 20 maximum degradation observed was EI of 16, higher than that observed for the detection of Tween 80 by esterases (EI = 7) (Fig 3 and Fig 4).

The production of lipases and esterases is a typical characteristic of several microorganisms, and is influenced mainly by growth conditions. In the present study, isolates R18(6) and 3(3) were not able to degrade lipases and esterases, respectively. These results may be linked to the narrow incubation temperature range and/or specificity of substrates used. Some authors claim that these enzymes are substrate-specific and, thus, their activities vary with the triacylglycerol composition of oils used [43, 44]. According to Vieira et al. [45], olive oil contains high levels of oleic acid (63%), a substrate preferentially degraded by lipases.

Esterases play an essential role in the synthesis of some clinically important medical drugs. Esterases produced by *Trichosporon brassicae* and other microorganisms, such as *Rhodococcus* spp. and *Bacillus circulans* may produce large amounts of compounds with therapeutic applications. Esterases produced by *Pseudomonas* spp. produce drugs with anti-inflammatory properties, like ibuprofen [46]. For Biely et al. [47], the degradation of ethylene glycol and the hydrolysis of aspirin can be carried out by *Streptomyces lividans*.

In a study that characterized endophytic bacteria, isolated from plants of the Cerrado biome, presenting potential use in bioremediation, the most commonly detected isolates (*Bacillus cereus*, *Staphylococcus pasteuri* and *Pseudomonas* spp.) were able to promote plant growth and to degrade esterases, β-glucosidases, amylases and proteases. In the present study, protease detection based on casein and gelatin degradation varied with the substrates used. The presence of caseinase was confirmed for 65.21% of the endophytic bacteria incubated between 25°C and 30°C. Except for isolate 5(3), the other isolates presented EI higher than 1.0 but lower than 3.0 (Table 1). These results were lower than those obtained by Karanja et al. [20], who observed EI values between 3.0 and 7.4 in the proteolytic hydrolysis of low-fat milk by *Streptomyces* isolates incubated between 27.5ºC and 32.5ºC.

Azeredo et al. [48] reported that *Streptomyces* isolated from soil in the Cerrado biome were able to hydrolyze gelatin, casein and bovine albumin, which is commonly observed in these microorganisms. In the present study, 34% of isolates hydrolyzed gelatin when grown at 30°C, while only 17.39% presented activity when incubated at 25°C or 28°C (Table 1). These values are lower than those found by Rodrigues [22] who observed the degradation of gelatin by 40% of actinobacteria incubated at 35ºC. Goshev et al. [49] observed similar results for thermophile actinomycetes, whose proteolytic activity fell when the temperature rose from 60ºC to 70ºC.

Catalase was produced by all isolates used in the present study, independently of incubation temperature. In a previous study, Sousa [23] observed that all *Streptomyces* isolates produced lipase, amylase and catalase, which play important roles in growth promotion and biological control of plant diseases. Some actinomycetes, like *Streptomyces coelicolor*, produce three different catalases [50] as a protection mechanism against osmotic and oxidative stress. The most important (*cat*A) is induced by hydrogen peroxide.

Here, some of the actinobacteria tested were proven to be potential producers of extracellular enzymes; however, only isolate R26(6) was able to degrade all substrates used. Catalase, lipase, esterase, pectinase and starch were produced by a considerable number of isolates (86.95%), which exhibited mean EI between 2.9 and 7.5. Still, only a few isolates degraded casein, carboxymethylcellulose and gelatin. Actinobacteria play an important role in the sustainability of ecosystems in the natural environment. These organisms, due to the ability to produce antibiotics and several enzymes, have a wide array of industrial applications. The high efficiency and selectivity of reactions they catalyze are important features in industrial processes, which make these microorganisms an

attractive choice, fostering further investigations in the attempt to find new sources of actinomycetes. In this sense, the results obtained in the present study underline the biotechnological potential of some of these isolates, and may add to the development of new, efficient industrial processes in sectors these microorganisms are useful.

5. ACKNOWLWDGEMENTS

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