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Production of Enzymes by Actinobacteria from Agricultural Areas of the Brazilian Semi-Arid Region

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Abstract
Actinobacteria are microorganisms that are widely distributed in nature. This bacteria phylum can be found in oceans but the rhizosphere is their main habitat. Since these microorganisms are also found in the Brazilian Semi-arid Region and are of great industrial and agronomic interest, the present study aimed to characterize the extracellular production profile of cellulase, amylase and xylanase in actinobacteria found in agricultural soils from that region. Cellulolytic activity was observed in 60.71% of the analyzed strains. All strains showed amylase production. The TUB 18 strain stood out with high values of the amylolytic (6.03) and cellulolytic (12.32) enzymatic index. Xylan halo of degradation was observed in 82.14% of the strains, where strains TUB 7 and TUB 8 stood out with the highest enzymatic index. Studies like this are important to obtain an overview of the genetic potential of microorganisms from Semi-Arid Regions, which can originate new promising industrial or agricultural products.

Keywords: Caatinga; Extracellular enzymes; actinobacteria; Streptomyces.

1. Introduction
The vast majority of microbial diversity is found in terrestrial habitats, especially in soil. The microbial communities present in the soil are responsible for maintaining ecosystem services, which are essential for the conservation of soil characteristics [1]. Soil microorganisms are important because they participate in most biogeochemical cycles, such as nitrogen cycling and the transformation of organic matter. They are also part of biotechnological processes, being sources of new substances and other metabolites of industrial interest and can be promoters of bioremediation and biocontrol [2].
Although the lignocellulosic components are the most abundant in plant biomass, they represent compounds that are difficult to degrade [3]. Thus, microorganisms, such as actinobacteria, that produce enzymes capable of degrading these complex natural compounds, become relevant in the sustainability of the ecological system [4]. Complex molecules present in the soil such as cellulose, starch, hemicellulose and humus can be hydrolyzed by enzymes such as cellulase, amylase and xylanase, which mineralize these nutrients making them available in the soil [5]. Several studies of actinobacteria from the Brazilian caatinga report the production of these enzymes [6] [7].
Given the importance of actinobacteria, the present study aimed to carry out the enzymatic characterization of actinobacteria from agricultural areas of Ubajara (Ceará State - Brazil). This research can be used as scientific support for future work on these microorganisms.

2. Materials and methods
Twenty-eight strains of actinobacteria were chosen from the culture collection of the Laboratory of Environmental Microbiology of the Federal University of Ceará (UFC). Strains were named TUB and numbered from 2 to 30 (TUB-2, TUB-3, TUB-4, etc.). To evaluate the hydrolysis capacity of cellulose, starch and xylan, tests were carried out where each of the 28 strains of actinobacteria was inoculated in duplicate in the form of four spots

in each Petri Dish with culture medium containing carboxymethylcellulose (CMC), starch, and xylan, respectively, as a carbon source. After inoculation, the Petri Dishes remained in a BOD at 28 ± 2 °C for 10 days. After this incubation time, the diameter (in millimeters) of each of the colonies formed by the actinobacteria colonies was measured.

To reveal the hydrolysis zone of the cellulolytic and amylolytic activities, 10 ml of 0.5% Congo red solution was added to the plates for 15 minutes at room temperature. After this time, the excess solution was discarded and 10 ml of NaCl (2M) were added to each plate, allowing it to react for 30 minutes at room temperature. After the saline solution, the presence and absence of hydrolysis halos were observed around the colonies. For the observation of the starch hydrolysis zone, 10 mL of lugol was pipetted and then it was possible to observe whether there was discoloration of the medium with the dye due to the hydrolysis of the starch, forming a very contrasting clear halo around the colony. The diameter of all formed halos was then measured [8] [5].

Enzymatic activity was determined by the enzymatic index (EI) that relates the diameters of the halo degradation and the colony, expressed in equation [9]:

$$EI = Dh / Dc$$

Dh is the diameter in mm of the hydrolysis halo and Dc is the diameter in mm of the actinobacteria colony. The results of the enzymatic tests were submitted to the analysis of variance in SISVAR version 5.6 and, subsequently, to the Tukey mean test.

3. Results & Discussion

The ability to produce hydrolytic enzymes is dependent on the habitat in which the actinobacteria are inserted [10]. Enzyme activity has already been recorded in soils of the Brazilian Caatinga [8], the Amazon [11], the Brazilian Cerrado [12] and the Atlantic Forest [13]. In this context, the role of actinobacteria in the carbon cycle is highlighted, having the ability to hydrolyze different carbon sources in the most diverse ecosystems.

The focus of this work was to evaluate the production of hydrolytic enzymes by actinobacteria isolated from agriculture areas of the Brazilian semi-arid region. The enzyme index is shown in Table 1, while Figure 1 illustrates these results by dividing the strains into different groups, associating them with a heatmap of their EI.

Table 1: Enzymatic index of actinobacteria regarding the production of cellulase, amylase and xylanase.

Strain	Enzymatic Index		
	Cellulase	Xylanase	Amylase
TUB-2	-	2,89	4,64
TUB-3	-	-	2,72
TUB-4	1,95	3,04	2,72
TUB-5	-	4,95	3,84
TUB-6	-	-	2,35
TUB-7	5,98	8,89	5,15
TUB-8	5,32	8,56	5,48
TUB-9	2,82	4,16	3,65
TUB-11	2,07	2,94	2,78
TUB-12	1,49	5,06	4,45
TUB-13	4,45	6,49	4,37
TUB-14	-	-	1,58
TUB-15	8,18	6,49	4,76
TUB-16	4,04	5,84	4,82
TUB-17	8,6	7	5,49
TUB-18	12,32	6,77	6,03
TUB-19	-	4,51	3,32
TUB-20	1,92	3,09	2,94
TUB-21	-	4,95	3,14
TUB-22	-	-	1,96
TUB-23	2,32	4,12	2,18
TUB-24	2,07	2,41	1,91
TUB-25	-	1,39	3,52
TUB-26	2,94	3,69	2,76
TUB-27	3,08	3,59	2,49
TUB-28	-	-	4,07
TUB-29	-	2,96	2,95
TUB-30	2,33	4,67	3,6

(-) No halo formation.
Source: Authors, 2022.

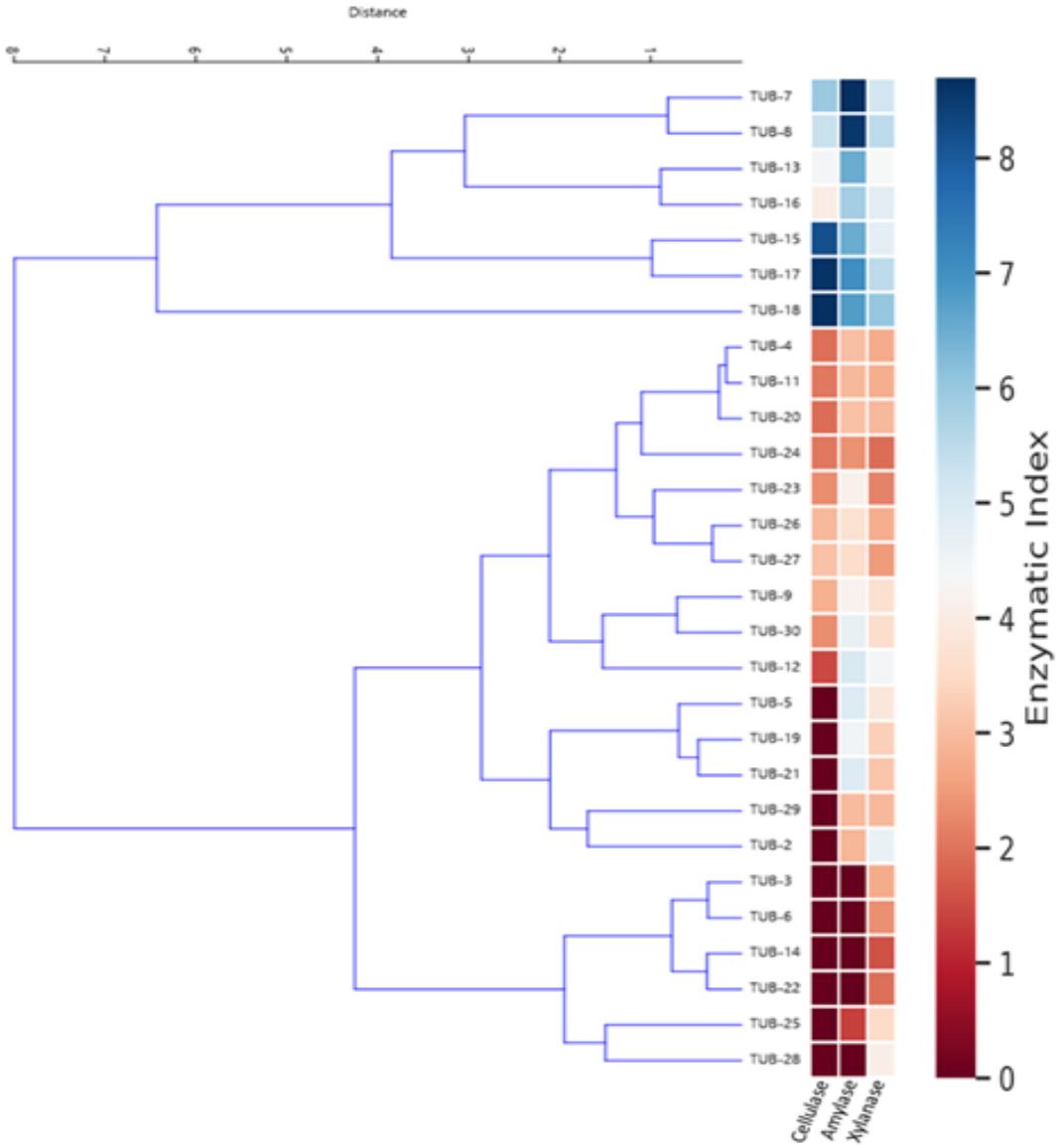


Fig. 1: Similarity dendrogram of actinobacteria associated with heatmap of their cellulolytic, amylolytic and xylanolytic enzymatic indices. Source: Authors, 2022

The cellulolytic activity indicator halo (Figure 2a) was observed in 17 strains, 60.71% of the analyzed strains. The indicator halo of starch degradation (Figure 2b) was observed in 100% of the analyzed strains (28), indicating that all are amylase producers. The clearest zone around the colonies, corresponding to the indicator halo of xylan degradation (Figure 2c), was observed in 23 strains (82.14%). In approximately 18% of the strains, the formation of an enzyme degradation halo was not observed, indicating that they do not produce xylanase.

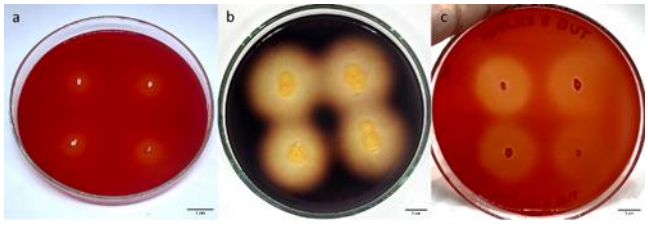
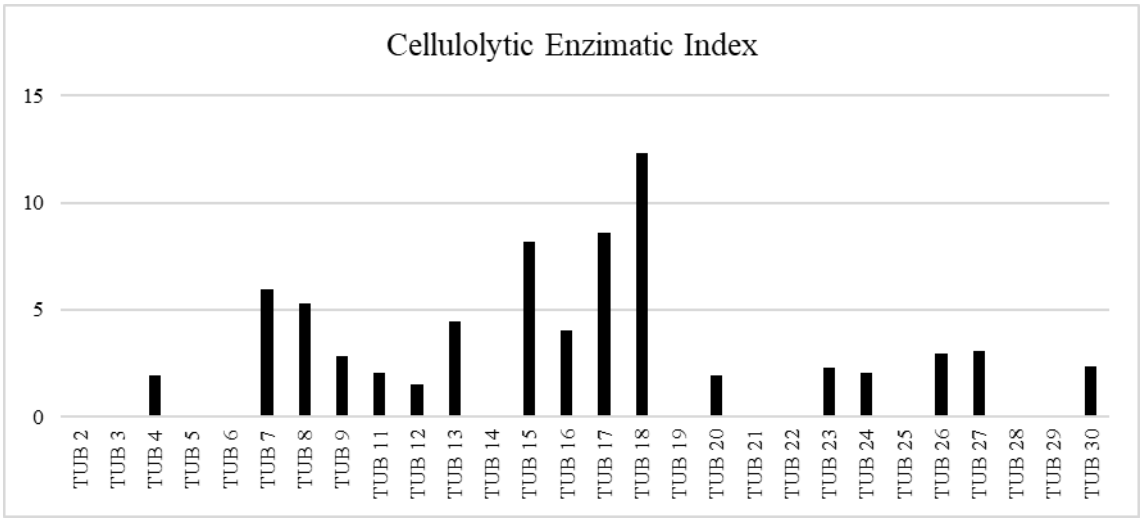


Fig. 2: Degradation halos of the strain TUB-18 in CMC medium (a), TUB-2 in starch medium (b), and TUB-8 in xylan medium (c). Source: Authors, 2022.

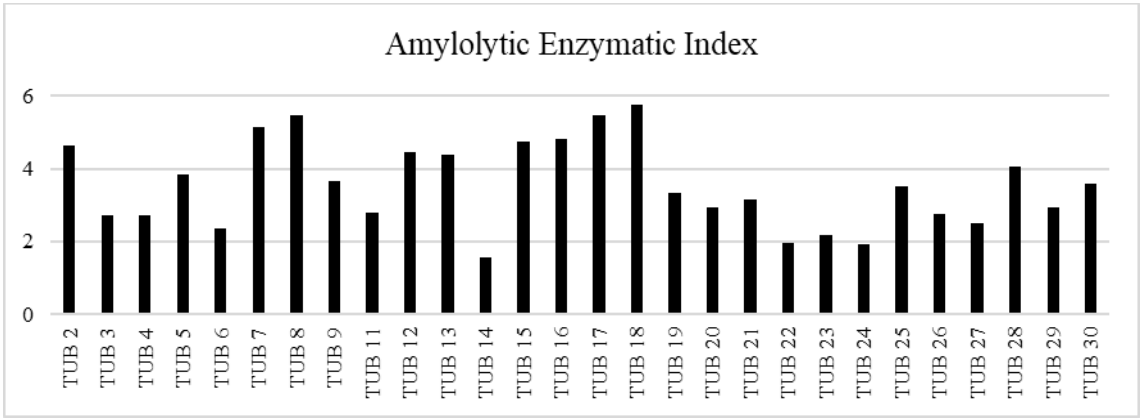
Silva et al. (2015) evaluating 28 strains of actinobacteria from the Ubajara National Park (Ceará, Brazil), reported cellulolytic activity at 75%, a value higher than that of the present study. This was probably due to the fact that our soil samples came from agricultural areas, which areas subjected to anthropic stress.

The enzymatic index of the 17 cellulase positive actinobacteria strains showed significant variations ($p<0.05$). The TUB 18 strain stood out with the highest EI, corresponding to 12.32, while the TUB 12 strain had the lowest EI value, 1.49, respectively (Graph 1).



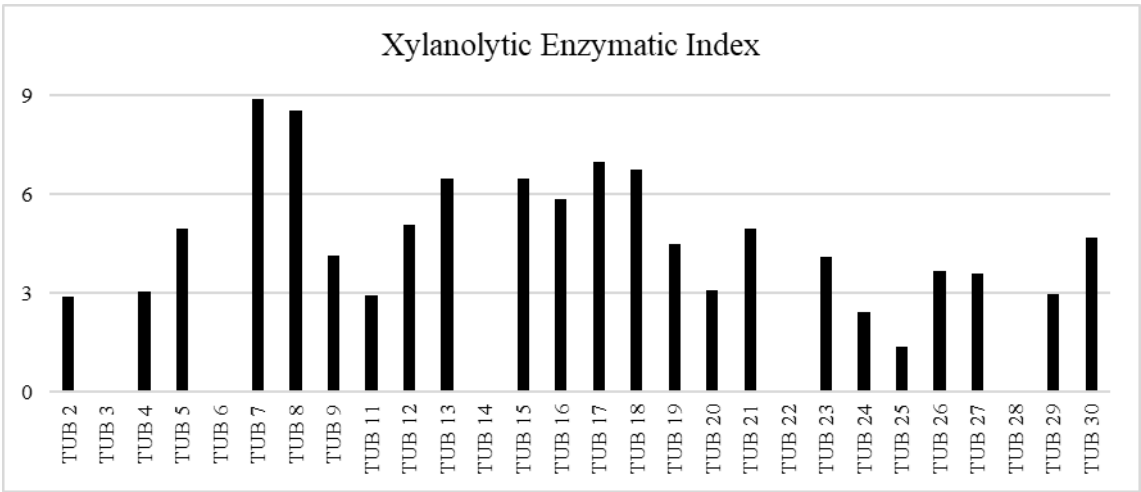
GRAPH 1 - Cellulolytic index of actinobacteria.
Source: Authors, 2022.

The enzymatic index of the 28 amylase positive actinobacteria strains showed significant variations ($p<0.05$). The TUB 18 strain stood out with the highest EI, corresponding to 6.03, while the TUB 14 strain had the lowest EI value (1.58) (Graph 2).



GRAPH 2 - Amylolytic index of actinobacteria
Source: Authors, 2022.

Xylanolytic activity is an essential factor in the decomposition of crop residues, since xylan is an abundant polysaccharide on the plant cell wall [14]. The enzymatic index of the 23 xylanase-positive actinobacteria strains showed significant variations ($p<0.05$). Strains TUB 7 and TUB 8, which did not differ statistically from each other, stood out with the highest indices, 8.89 and 8.56, respectively, while strain TUB 25 had the lowest EI value of 1.39 (Graph 3).



GRAPH 3 - Xylanolytic index of actinobacteria
Source: Authors, 2022.

Sousa et al. (2018) studying 38 strains of actinobacteria from Quixadá (Ceará - Brazil) obtained the maximum xylanolytic index value of 3.02, while the highest EI found in this study is 8.89 and 8.56, much higher values.

4. Conclusion

The semiarid agricultural areas of Brazil have an immeasurable microbial diversity. The microorganisms in this region have great biotechnological potential, which reinforces the importance of prospecting studies like this one. The images obtained, as well as the integration of the methodologies used, can help in future work on this topic.

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References

1. HUANG, Hanyue et al. Effects of afforestation on soil microbial diversity and enzyme activity: A meta-analysis. Geoderma, v. 423, p. 115961, 2022.
2. CHUKEATIROTE, Ekachai; PHUEAOUAN, Thanong; PIWKAM, Anong. Screening of rhizosphere soil bacteria for biocontrol of Lasiodiplodia theobromae. Agriculture and Natural Resources, v. 52, n. 4, p. 325-329, 2018.
3. Irfan, M. et al. Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity. Türk Biyokimya Dergisi / Turkish
4. Journal of Biochemistry, 2012; 37: 120-128.
5. Baldrian, P. Distribution of extracellular enzymes in soils: spatial heterogeneity and determining factors at various scales. Soil Science Society of America Journal, 2014; 78: 11-18.
6. Burns, R. G. et al. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biology and Biochemistry, 2013; 58: 216-234.
7. Silva, G. R. Bioprospecção de actinobactérias isoladas da rizosfera de Caesalpinia pyramidalis Tul. do bioma Caatinga. Dissertation - Universidade Federal de Pernambuco, Recife. 2013.
8. Sousa, J. B., et al. Effect of antropization on the production of xylanase enzyme of actinobateria. Enciclopédia Biosfera, 2018; 15(28): 1033-1042.
9. Alves, D. et al. Production cellulase and amylase of actinobacteria from Brazilian Semiarid. Enciclopédia Biosfera, 2016; 13(24): 1303-1315.
10. Hankin, L.; Anagnostakis, S. L. Solid media containing carboxymethylcellulose to detect cellulose activity of micro-organisms. Journal of General Microbiology, 1977; 98(1): 109-115.
11. Sakure, S. et al. Isolation and characterization of Actinomycetes from rhizosphere soil of different plants for antiphytopathogenic activity and stress tolerance. International Journal of Current Microbiology and Applied Sciences, 2015; 2: 379-387.
12. Oliveira, R. L. Production and Characterization cellulases Extra-Cell Produced by Actinomycetes. Thesis - Universidade Federal do Amazonas, Manaus. 2015.
13. Silva, G. R. Bioprospecção de actinobactérias isoladas da rizosfera de Caesalpinia pyramidalis Tul. do bioma Caatinga. Dissertation - Universidade Federal de Pernambuco, Recife. 2013.
14. Jesus, J. A. Biotechnological potential of actinobacterias and endophytic diazotrophic bacterias for plant growth. Dissertation - Universidade Estadual do Norte Fluminense
15. Darcy Ribeiro, Campos dos Goytacazes. 2013.