EPIPHYTIC BACTERIA OF BROMELIAS FROM CHAPADA DIAMANTINA AND THEIR CELLULOLYTIC POTENTIAL

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ABSTRACT

Chapada Diamantina, located in Bahia, has a unique plant diversity. The region's climatic adversities confer a high level of endemism of fauna, flora and microorganisms, selecting individuals adapted to local conditions. Bromeliads are a biodiversity hotspot, in addition to having the capacity for epiphytism, where, through the trichomes present on their leaves, they can absorb water and nutrients and also have the presence of microorganisms that colonize the phylloplane and play fundamental roles in promoting and protecting from your host plant. Swabs soaked in a solution of 0.85% NaCl and 0.3% Tween 80 were lightly rubbed on the leaf surface of bromeliads, processed and inoculated in Trypticasein Soy Agar (TSA) medium for bacterial isolation, followed by incubation. Selected morphologically distinct bacterial colonies were purified and cryopreserved. Active strains were selected for cellulolytic activity on CMC agar medium, with subsequent statistical analysis of the data. A total of 104 isolates were tested, the analyzes revealed that 74 strains (71.15%) are capable of producing the hydrolytic enzyme cellulase. With the semiquantitative test, five isolates showed a high enzyme index, ranging from 2.07 to 3.02. Evidencing the bacteria from the Bromeliad phylloplane as promising in the production of hydrolytic enzymes of industrial interest

Keywords: Biotechnology. Semi-arid. Microbiology. Enzymes.

1 INTRODUCTION

The Caatinga, with its diversity of plant species adapted to the extreme conditions of the semi-arid northeast, is a biome that has a rich variety of microorganisms, many of which actively colonize plants, both on their surfaces and in their internal tissues[1]. This phenomenon is particularly evident in bromeliads, which not only offer unique ecological niches such as water tanks and rosette-shaped leaves[2], but also have a peculiar capacity for extreme epiphytism[3]. These plants, especially phytotelmata bromeliads, absorb water and nutrients directly from the leaves through trichomes, instead of depending on the root system[4], taking advantage of the surrounding decomposing residues. The phylloplane, leaf surface, of plants, a heterogeneous but essential part, is colonized by a variety of microorganisms that have adapted to challenging conditions, such as high exposure to UV radiation, extreme temperatures and low water availability[5].

In this context, bacteria emerge as active colonizers of the phylloplane, playing important roles in plant ecology[5]. In addition to their known potential in disease and pest control, bacteria and their metabolites, including extracellular enzymes, are increasingly being explored for diverse biotechnological applications. Tropical regions, such as the Caatinga Biome and structures found in bromeliads, offer a hotspot of microbial biodiversity with biotechnological potential, including those capable of producing valuable enzymes, such as cellulase[6,7]. The performance of these enzymes is crucial for bromeliads, as they participate in the process of decomposing the leaves that fall into their tanks and surrounding areas, improving the nutritional availability of this microhabitat, as is the case of cellulase, which decomposes the cellulose present in plant leaves[8].

Thus, although there are studies on bacteria associated with bromeliads, the potential of these microorganisms for the production of industrial enzymes is promising, motivating studies along these lines, which aim to characterize the epiphytic isolates of bromeliads and describe the bacterial community associated with the phylloplane, in addition to evaluating the potential of isolated bacteria for the production of enzymes of industrial interest.

2 MATERIALS & METHODS

To prospect for bacterial isolates, areas of vegetation were chosen in the Chapada Diamantina region, within the Caatinga biome. Using swabs moistened in a solution of 0.85% NaCl and 0.3% Tween 80, samples of the material contained in the phylloplane of bromeliaceae (Bromeliaceae Juss.) were collected by rubbing the swabs over the entire surface. The samples were then deposited in tubes containing 0.85% NaCl solution. They were then subjected to serial dilutions in 0.85% NaCl solution and inoculated in Trypticasein Soy Agar (TSA) and incubated at 28 ± 2°C for 72 hours. After the incubation period, morphologically distinct bacterial colonies were selected and purified, which were subsequently cryopreserved in 50% glycerol at -20°C, composing the bacteriological collection of semi-arid microorganisms at the Semiarid Microbial Ecology and Biotechnology Laboratory (SMEBIL).

The purified bacterial isolates were inoculated in Trypticasein Soy Agar (TSA) medium and incubated at 28 °C for 24 hours in a bacteriological growth oven. The active strains were inoculated for cellulolytic activity screening in CMC agar medium (g/L) (10 carboxymethylcellulose (CMC),0,2 $MgSO_4$. $7H_2O$; $4KH_2PO_4$; 0,004 $FeSO_4$. $7H_2O$; 0,001 $CaCl_2$. $2H_2O$; 4 Na_2HPO_4; 4 of yeast extract e 15g agar with pH 6)[9], followed by incubation at 28 °C for 72h. After incubation, the CMC agar medium was washed with 5 mL of 0.1% Congo red reagent for 15 minutes, followed by washing with 1 M NaCl. The presence of a clear zone around the colony indicates cellulolytic activity by the isolate.

To determine the enzymatic index (IE) of the cellulolytic isolates, a semiquantitative test was carried out, using the previously mentioned parameters of medium, time, temperature and halo development. The enzymatic index (IE) was determined based on measurements of halo diameter - HD (mm) and colony diameter - CD (mm), measured using a caliper[10]. The strains were classified according to their enzymatic index, where IE values ≥ 2 have high productivity, strains with 1.5 ≤ IE < 2.0 have moderate productivity and isolates with 1.0 ≤ IE < 1.5 are weakly producing[11].

$$\text{Enzymatic index} = \frac{HD}{CD}$$

The enzyme activity assay was carried out in triplicate, considering each plate as an experimental unit. The EI data were analyzed based on one-way ANOVA, when significant variations were found, the strains were compared with each other using the Scott-Knott test ($p<0.05$), using the AgroEstat program.

3 RESULTS & DISCUSSION

The cellulose supplemented to the medium will be hydrolyzed due to the action of the cellulase enzyme that is produced by the bacteria. In this process, a clear zone appears in the middle, which is revealed after contact with Congo red dye, which reacts with the 1,4-glycosidic substance contained in the cellulose polymer[12]. The orange halo around the colonies indicates the level of enzyme activity and consequent cellulose degradation[13].

In screening the isolates, of the 104 strains tested, 74 strains (71.15%) were identified as being capable of hydrolyzing cellulose. In the semiquantitative test (Figure 1), five isolates showed greater potential 103, 41, 21, 23, 06 with high EI, ranging from 2.07 to 3.02, respectively. While, seven isolates with moderate EI, EI between 1.75 and 1.50, and 62 with low EI, with values equal to or lower than 1.50 (Table 1).

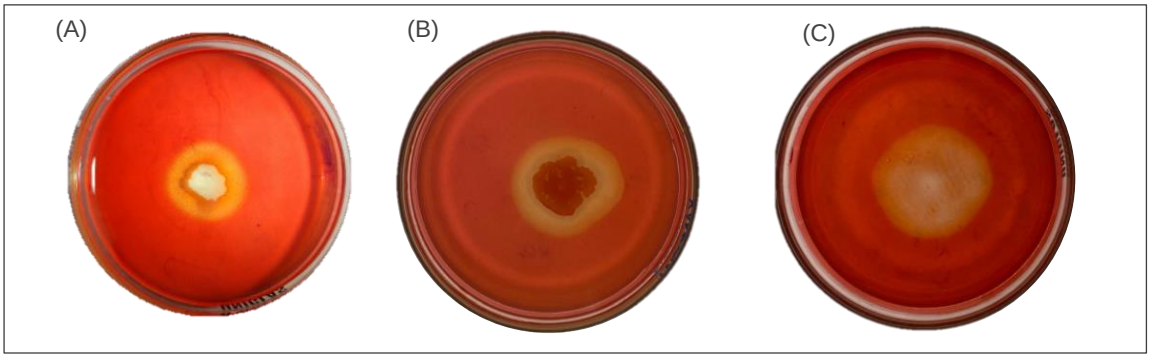

Figure 1 Production of cellulases by (A) 103, (B) 41 and (C) 06, isolated from the phylloplane of Bromeliads spp.

Table 1 Enzymatic index (IE) of cellulase of isolates from Chapada Diamantina National Park cultivated in CMC Agar medium at 28°C for 72h.

Isolated	Enzymatic index	Classification of Enzyme activity	Isolation location
06	3,02 a	High	Philoplan
23	2,23 b	High	Philoplan
21	2,83 b	High	Philoplan
41	2,08 b	High	Philoplan
103	2,07 b	High	Philoplan

The results correspond to the average of the triplicates. Means followed by the same letter in the column do not differ from each other using the Scott-Knott test at 5% probability

The oscillation between enzymatic levels is due to the ability of each isolate to hydrolyze the cellulose contained in the medium, due to the secretion of the enzyme endo-β-1,4-glucanase (CMCase), produced by cellulolytic bacteria by breaking the glycosidic

bond ÿ -1.4 in CMC14 medium. Furthermore, the phyllosphere is an extreme, dynamic, heterogeneous and often oligotrophic[15] environment, thus shaping the region's microbial community. Cellulolytic microorganisms play a fundamental role in the decomposition of organic matter, the mineralization of nutrients and the promotion of plant growth. They can also act in the biological control of phytopathogens of agricultural interest, such as fungi of the genus *Phytophtora* and *Pytium*[16]. It is worth mentioning that the microbial community of the phyllosphere is regulated by the population processes of immigration, emigration, growth and death[17] and knowing the epiphytic capacity of Bromeliads, together with the cry for help mechanisms, the plant signals the need for microorganisms to perform functions specific, be it nutrition or protection, and can be performed by the same isolate, given its elite nature that allows it to perform several functions in association with a single host, or by isolates “invited” to perform such a function.

4 CONCLUSION

The epiphytic microbial communities of Caatinga plants emerge as a source of great biotechnological potential for the production of cellulolytic enzymes. The enzyme synthesis revealed in this study not only highlights the intrinsic value of the natural resources of Bahia's semi-arid region, but also highlights the urgency of further investigation in this area. This effort is essential to promote the development of sustainable alternatives in different industrial sectors.

REFERENCES

1 FONSECA, Á., INÁCIO, L. 2006. Phylloplane Yeasts. *In:*Biodiversity and Ecophysiology of Yeasts. PÉTER, G., ROSA, C., (ed) The Yeast Handbool. Berlin, Heidelberg. 263-301.
2 MESTRE, L.A.M., ARANHA, J.M.R., ESER, M.L.P. 2001. Braz Arch Biol Technol. 44(1). 89-94.
3 LOPEZ, L.C.S., ALVES, R.R.D., RIOS, R.I. 2009. Hydrolbiol. 625 (1). 151-156.
4 HUMBER, R.A. 2008. J. Invert. Pathol. 98 (1). 262-266.
5 VORHOLT, J. A. 2012. Nat. Rev. Microbiol.10 (12). 828-840.
6 Buzzini, P., MARTINI, A. 2002. J. Appl. Microbiol. 93 (6). 1020-1025.
7 SILVA, K., CASSETARI, A., S., LIMA, A. S., BRANDT, E., PINNOCK, E., VANDAMMEC, P., MORREIRA, F.M.S. 2012. Syst. Appl. Microbiol. 35 (1). 253-262.
8 GOMES, F.C.O., SAFAR, S.V.B., MARQUES, A.R., MEDEIROS, A.O., SANTOS, A.R.O., CARVALHO, C. LACHANCE, M.A., SAMPAIO, J.P., ROSA, C.A. 2015. Antonie van Leeuwenhoek.J.Microbiol.107 (2). 597-611.
9 KHOIRUNNISA, N.S., ANWAR, S., SANTOSA D.A. 2020. Biodivers. J. 21 (4). 1686-1689.
10 STAMFORD, T.L.M., ARAUJO, J.M., STAMFORD, N.P. 1998. 18 (1). Food. Sci. Technol. 18 (4). 382-385.
11 SILVA, V.M.A, BRITO, F.A.E.D., RAMOS, K.A., SILVA, R.M.D., MARTINS, C.M., MARTINS S.C.S. 2015. Rev. Bras. Geogr. Fís. 8 (1). 560-572.
12 ZVERLOVA, V.V., HOLL, W., SCHWARZ, H., 2003. Int. Biodeterior. Biodegradation. 51 (3). 175-179.
13 JO, W.S., PARQUE, H.N., CHO, D.H., YOO, Y.B., PARQUE, S.C. 2011. Mycobiology. 39 (2). 129-132.
14 WEERASINGHE, W.M.L.I., MADUSANKA, D.A.T., MANAGE, P.M. 2021. Int. J. Renew. Energy Dev. 10 (4). 699-711.
15 KOSKELLA, B. 2020. Curr. Biol. 10 (19). 1143-1146.
16 LIMA, L.H.C., DE MARCO, J.L., FELIX, C.R. 1998. Enzimas Hidrolíticas Envolvidas no Controle Biológico por Micoparasitismo. *In:* Controle Biológico. MELO, I.S., AZEVEDO, J.L. (ed) EMBRAPACNPMA. Jaguariúna, São Paulo. 263-301.
17 KINKEL, L. 1997. Annu. Rev. Phytopathol. 35 (1). 327-347.

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