PROSPECTING OF CELLULOLYTIC BACTERIA FROM THE PHYLLOSPHERE OF NATIVE CAATINGA PLANTS

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ABSTRACT

The Caatinga biome has associations between plants and microorganisms that can confer resistance to stress conditions to plant species. These microorganisms, in addition to their biological function during association, have high biotechnological potential due to the production of different components, such as cellulases. The aim of this work was to evaluate the presence of cellulase-producing bacteria isolated from the phyllosphere of endemic Caatinga plants. The microorganisms were isolated from the surface of cacti and bromeliads and inoculated into cellulase-inducing culture medium to evaluate enzyme production. The hydrolytic activity was verified by the formation of halos revealed by the addition of 0.1% Congo red to the plates and evaluated by the Enzymatic Index (IE). The data were analyzed using the Scott–Knott test at 5% significance. A total of 78 bacteria were isolated, 37 (47.44%) of which exhibited cellulolytic activity. Of these, 13, 7 and 17 showed high, moderate and low activities, respectively. These results show the biotechnological potential of natural resources in the semiarid region of Bahia and highlight the phylospheric environment as a source of new technologies for the industrial sector.

Keywords: Cellulase. Epiphytic. Holobiont. Semiarid.

1 INTRODUCTION

The interaction between plants and microorganisms provides a variety of benefits to host plant species, such as tolerance to abiotic stress conditions and protection against pathogens, in addition to providing microbial communities with adaptations resulting from coevolution between organisms[1]. This synergistic relationship, defined as a holobiont, also occurs in Caatinga, a biome characterized by irregular rainfall, high temperatures and vegetation adapted to extreme conditions[2].

In addition to adaptations to the water deficit of the semiarid climate, plant species in the biome establish relationships with microorganisms that promote tolerance to numerous stress conditions. The maintenance of this synergistic relationship is reflected in the synthesis of different compounds; the microbiome of Caatinga plant species is characterized by the production of metabolites and substances of biotechnological interest, such as phytohormones, antibiotics, and enzymes, which act as important biocatalysts in industry[3 4 5].

Cellulases (EC.3.2.1.) are the most prominent enzymes that perform fundamental hydrolytic activity by breaking the β (1→4) glycosidic bonds of cellulose, the most abundant polysaccharide on the planet. Widely synthesized by bacteria and fungi, these enzymes have attracted considerable biotechnological interest due to their applications in the textile and paper industry and food sector, as well as their use in the degradation of lignocellulosic waste to generate new energy sources[6].

Driven by the intensification of climate change and the depletion of fossil resources, the search for energy alternatives based on natural resources has become a priority for reducing anthropogenic impacts on the planet[7]. Lignocellulosic waste from agro-industrial processes has emerged as a promising raw material for the production of renewable energy, especially due to the possibility of converting its biomass through the hydrolysis of the polysaccharides that make up its structure, including cellulose[8].

In this scenario, the prospecting of microorganisms with cellulolytic activity plays a crucial role in the construction of new technologies for economic development aligned with sustainability. Thus, this study aimed to evaluate the presence of cellulase-producing bacteria isolated from the phyllosphere of plant species native to the Caatinga.

2 MATERIAL & METHODS

The prospecting of isolates was carried out in vegetation areas of the Caatinga biome located in the municipalities of Paulo Afonso (latitude: 9° 24' 39" S; longitude: 38° 14' 9" W), Jeremoabo (latitude: 10° 3 ′ 50″ S; longitude: 38° 20' 27" W), Glória (latitude: 9° 20' 45" S; longitude: 38° 16' 22" W) and Canudos (latitude: 11° 57' 57" S; longitude: 41° 37' 37" W). Swabs moistened in 0.85% NaCl and 0.3% Tween 80 solution were rubbed on the surface of cacti (Cactaceae Juss.) and bromeliads (Bromeliaceae Juss.) native to the Caatinga and deposited in tubes with 0.85% NaCl solution. The samples were serially diluted, inoculated in TSA

culture medium (tryptone soy agar) and incubated at 28 ± 2°C for 72 h. Morphologically distinct bacterial colonies were purified and cryopreserved at −20°C.

To evaluate the cellulolytic activity, each isolate was inoculated in triplicate in a culture medium composed of: (g/L) 15 agar, 4 KH_2PO_4, 4 yeast extract, 4 Na_2HPO_4, 0.2 $MgSO_4.7H_2O$, 0.004 $FeSO_4.7H_2O$, 0.001 $CaCl_2H2O$ and 1% sodium carboxymethylcellulose (CMC) as the sole carbon source (pH 6.0) [9]. After incubation for 72 h at 28 ± 2°C, a 0.1% Congo red solution was added to the plates and incubated for 15 min and rinsed with 1 M NaCl to reveal the hydrolysis zones. The relationship between the halos and the diameters of the bacterial colonies was used to determine the Enzymatic Index (EI) (1)[10]:

$$EI = \frac{halo\ diameter\ (mm)}{colony\ diameter\ (mm)} \quad (1)$$

The data were analyzed using the Scott–Knott test at 5% significance.

3 RESULTS & DISCUSSION

We obtained 78 bacteria isolated from the phyllosphere of species native to the Caatinga. Of these, 57 isolates (73.08%) were from cacti cladodes, while the other 21 (26.92%) were from the bromeliads phyllosphere. The isolates were purified and deposited in the microorganism collection of the Semiarid Microbial Ecology and Biotechnology Laboratory, Bahia State University (Campus VIII – Paulo Afonso).

Enzyme production, evaluated semiquantitatively by the relationship between the dimensions of halos and colonies (Figure 1), was observed in 37 isolates (47.44%). The enzyme index (EI) of the producing strains varied significantly between 1.03 and 3.42 (Table 1), corroborating previously reported data demonstrating that the Caatinga microbiome is a source of microbial enzymes with cellulolytic activity [11 12]

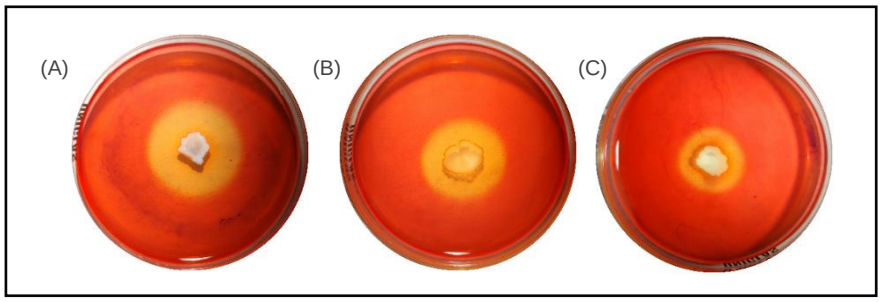

Figure 1 Production of cellulases by (A) RPM2, (B) BQP1 and (C) BMB8, which were isolated from the surface of Tacinga palmadora (Britton & Rose) N.P. Taylor & Stuppy, Tacinga inamoena (K. Schum.) N.P. Taylor & Stuppy and Bromelia laciniosa Mart. ex Schult. & Schult.f., respectively.

Table 1 Enzyme Index (EI) of cellulase-producing isolates prospected from the surface of native Caatinga plants. Means followed by the same letter do not differ from each other according the Scott-Knott test at 5% significance ($p < 0.05$)

Strain	EI	Strain	EI	Strain	EI	Strain	EI
BCF3	1,12 ± 0,15 c	BXX1	2,78 ± 0,20 a	SCF3	1,58 ± 0,19 c	UMB6	1,36 ± 0,02 c
BCF4	1,60 ± 0,03 c	ECH2	2,84 ± 0,36 a	SXX1	1,30 ± 0,22 c	UMD1	1,24 ± 0,11 c
BGR2	1,87 ± 0,53 c	EQP3	1,16 ± 0,08 c	SPM2	2,53 ± 0,92 b	UQP2	1,32 ± 0,10 c
BMB1	1,55 ± 0,65 c	EQP4	1,27 ± 0,30 c	SPM3	1,22 ± 0,17 c	UQP3	1,03 ± 0,04 c
BMB2	2,70 ± 0,12 a	ERR1	1,52 ± 0,06 c	SPM6	3,43 ± 0,20 a	UXX1	2,39 ± 0,45 b
BMB3	1,85 ± 0,43 c	PCF1	1,64 ± 0,38 c	SPM7	2,94 ± 0,29 a	UXX2	1,14 ± 0,09 c
BMB6	1,35 ± 0,18 c	PMB1	1,09 ± 0,01 c	UFX1	2,91 ± 0,53 a	UXX3	2,12 ± 0,27 b
BMB7	1,40 ± 0,39 c	RFX3	1,19 ± 0,19 c	UMB2	2,43 ± 0,89 b		
BMB8	1,72 ± 0,16 c	RPM2	2,53 ± 0,38 b	UMB3	2,06 ± 0,60 b		
BQP1	2,03 ± 0,19 b	SCF1	1,31 ± 0,15 c	UMB5	1,43 ± 0,03 c		

According to the classification of enzyme production proposed by Silva et al.[11], of the 37 producing isolates, 17 strains (45.95%) exhibited low cellulase synthesis ($1.0 \leq EI < 1.5$), moderate cellulase production ($1.5 \leq EI < 2.0$) was detected in 7 strains (18.92%), while high cellulase production ($EI \geq 2.0$) was detected in 13 strains (35.13%).

In relation to strains with high cellulase production ($IE \geq 2.0$), there was significant production of prospected cactus isolates; among the 13 isolates, 10 strains (76.92%) were isolated from the surface of the cladodes of *Hylocereus setaceus* (Salm-Dyck) Ralf

Bauer), *Pilosocereus pachycladlls* Ritter, *Tacinga palmadora* (Britton & Rose) N.P. Taylor & Stuppy, *Tacinga inamoena* (K. Schum.) N.P. Taylor & Stuppy and *Pilosocereus gounellei* (F.A.C. Weber) Byles & Rowley. The production of cellulases by bacteria associated with cacti was reported by Kavamura et al.[13], who demonstrated high cellulolytic activity (EI ≥ 4.0) in *Bacillus* spp. strains isolated from *Cereus jamacaru* D.C., *Melocactus* spp. and *P. gounellei*. In this context, the cacti-associated microbiome stands out as viable source for the devenlopment of new technologies.

Three strains (23.08%) among the isolates with high cellulolytic activity (IE ≥ 2.0) were from bromeliads (*Bromelia laciniosa* Mart. ex Schult. & Schult.f.). Mautone et al.[14], when evaluating the production of enzymes that degrade the plant cell wall by epiphytic yeasts from bromeliads, highlighted the high potential of the phyllosphere as a source of enzymes of industrial interest.

The presence of cellulolytic bacteria in the phylospheric environment reveals the importance of the participation of microorganisms related to the carbon cycle; the ability to degrade the lignocellulosic matrix provides microbial communities with an abundant nutritional source adaptation to oligotrophic conditions[15 16]. The coevolution between plants and phyllosphere microorganisms can promote cooperation to mitigate biotic and abiotic stresses. Plant species, through a mechanism called cry for help, recruit microbial communities that produce compounds that help them resist various stress conditions, such as pressure from phytopathogens[17]. In this context, the stress conditions detected in the Caatinga biome may be associated with the high cellulolytic activity reported in the present study.

The prospecting of cellulolytic bacteria from the phyllosphere, whose application in the biological control of phytopathogenic fungi has been reported in the literature[18], can be justified by the recruitment of microorganisms by cacti and bromeliads. Exposure to semiarid climate conditions can favor the presence and dominance of microbial communities that produce compounds and metabolites, including cellulases, which mitigate the effects of different stresses on plant species.

4 CONCLUSION

The epiphytic microbial communities of Caatinga plants have potential for the production of cellulolytic enzymes. The enzymatic synthesis demonstrated in this study highlights the importance of natural resources in the semiarid region of Bahia and highlights the need to deepen knowledge in this area to encourage the development of sustainable alternatives for the different sectors of the industry.

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