CELLULOLYTIC ACTIVITY OF RHIZOBACTERIA ASSOCIATED WITH SISAL (*Agave sisalana* Perrine ex Engelm.)

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ABSTRACT

Bacterial cellulases are biocatalysts of biotechnological interest due to their ability to convert cellulose into industrial sugars, which are potentially usable in the production of biofuels. This study aimed to isolate and evaluate bacteria from the Sisal rhizosphere with hydrolytic activity for cellulose. The bacteria were isolated from the sisal rhizosphere and inoculated into cellulase-inducing culture medium to evaluate enzyme production. Enzymatic activity was quantified using the enzymatic index (EI) based on the formation of halos around the colonies after the addition of 0.1% Congo red solution to the culture medium. The data were subjected to analysis of variance (ANOVA) using the Scott–Knoot test at 5% significance. A total of 137 bacteria were isolated, 40 (29.20%) of which exhibited enzymatic activity, as confirmed by the formation of hydrolysis zones around the colonies. Among the isolates, 18, 9 and 13 exhibited high, moderate and low enzymatic productivity, respectively. These results show that bacteria from the sisal rhizosphere have cellulolytic activity and can be evaluated for their potential use in industry. However, additional studies are needed to elucidate the synthesis and enzymatic activity of rhizobacteria.

Keywords: Biofuels. Cellulase. Semiarid. Industry.

1 INTRODUCTION

Sisal (*Agave sisalana* Perrine ex Engelm.) is a species native to Mexico and the southwestern United States and stands out as a viable alternative for fiber production due to its versatility in textile and industrial applications, mainly due to its high productivity and low cost of production[1]. The relevance of the crop is also related to its potential for use as a raw material for biofuels due to its lignin content, ease of decomposition, and high production yields.

In Brazil, sisal production is predominant in the northeast region, which is characterized by the Caatinga biome and semiarid climatic conditions. In this environment, plants of the Agave genus are adapted, reducing competition for land and agricultural crops[2,3]. This adaptability to abiotic stress in the semiarid region is partly due to the association between microorganisms and plants, which share a process of coevolution, ensuring beneficial relationships in the phyllosphere, endosphere and rhizosphere.

The microorganisms present in the root system provide several crucial compounds to plants, such as phytohormones, which can induce tolerance to biotic and abiotic conditions. Among these microorganisms, cellulolytic bacteria are of great interest in both agriculture and industry due to their ability to degrade cellulose, a renewable resource with great potential for bioconversion into bioproducts with high economic impact[4].

Cellulose is a polysaccharide that structurally constitutes the cell walls of plants in pure form or combined with lignins and hemicelluloses[5]. It has a crystalline portion that is insoluble in organic and alkaline solvents at room temperature[6,7] and is an economical alternative for several industrial applications, including the production of biopolymers and second-generation biofuels.

However, the bioconversion of cellulose into fermentable sugars is based on the reaction of multiple enzymes for hydrolysis, increasing the cost of the process[8]. Biocatalysts from bacterial consortia can synthesize several enzymes, such as endoglucanases, exoglucanases, d-cellodextrinases, cellobiohydrolases and β-glucosidase, which participate in the enzymatic hydrolysis of cellulosic biomass, resulting in the production of fermentable sugars and subsequent production of biofuels[9]. This contributes to reducing production costs and accelerating the cellulose degradation process.

Furthermore, cellulases, which are used in animal nutrition, brewing and the production of biofuels, are of great industrial interest. Therefore, studies on the enzymatic potential of microorganisms as an important technology for their bioconversion activity and generation of renewable energy have become crucial. Bacteria-synthesized cellulases are environmentally friendly and efficient alternatives to conventional physical and chemical methods due to the specificity of the reaction, conditions and enzyme production. This work aimed to isolate and evaluate the cellulolytic activity of bacteria from the Sisal rhizosphere.

2 MATERIAL & METHODS

Sisal rhizospheric soil samples were collected in the Alto Bonito Settlement, located in the municipality of Cansanção (Latitude: 10° 40 15' S; Longitude: 39° 29' 57 W), in Bahia. Subsequently, the samples were sent to the Semiarid Microbial Ecology and Biotechnology Laboratory at the State University of Bahia, *Campus* VIII, Paulo Afonso-BA.

Ten grams of soil sample was added to 90 mL of saline solution (0.85%) and stirred at 150 RPM for 40 minutes. The suspension was serially diluted, and a 0.1 mL aliquot was inoculated into TSA culture medium ((g/L-1) tryptone 15.0; soybean papaya digestion 5.0; sodium chloride 5.0; agar 15.0). The plates were kept in a bacteriological oven at 28 ± 2 °C for 48 hours. Morphologically distinct colonies were purified and preserved in glycerol (50%) at -20 °C.

The bacterial isolates were inoculated in triplicate on CMC agar medium ((g L^{-1})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 $CaCl_2.2H_2O$ and 1% carboxymethylcellulose, pH 6.0)[10]. The plates were incubated for 72 h at 28 ± 2 °C, after which a Congo red solution (0.1%) was added to the culture medium and incubated for 15 min. Subsequently, the plates were rinsed with saline solution (1 M) for 10 min to reveal the hydrolysis halo. The enzymatic index was determined according to Hankin and Anagnostakis[11] as follows (1):

$$E.I. = \frac{\emptyset\ halo(mm)}{\emptyset\ colony\ (mm)} \quad (1)$$

The data obtained were subjected to a variance test (ANOVA) with the Scott–Knott test at 5% significance using AgroEstat software version 1.1.

3 RESULTS & DISCUSSION

From the rhizospheric sisal soil samples, 137 morphologically distinct bacteria were isolated. Among these, enzymatic activity was confirmed for 40 (29.20%) bacteria, which showed the formation of hydrolysis zones around the colonies. Among the isolates CR96, CR111 and CR37 produced the highest levels of these enzymes (**Figure 1**).

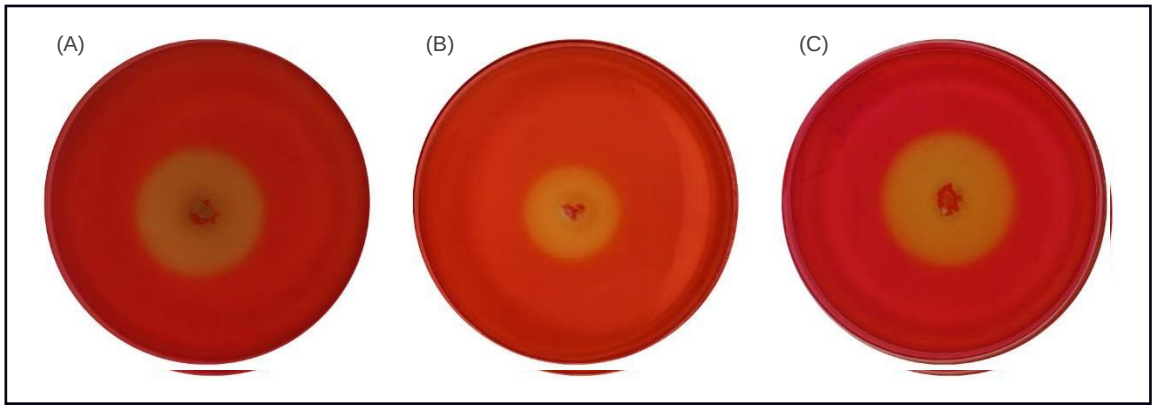

Figure 1 Enzymatic hydrolysis by the (A) CR96, (B) CR111 and (C) CR37 isolates with high enzymatic indices (EIs).

The enzymatic index ranged from 1.01 (low) to 5.57 (high), based on the classification proposed by Silva et al.[12], where 13 (32.5%) bacteria demonstrated a low enzymatic index (1.0 ≤ IE <;1.5), 9 (22.5%) had moderate production (1.5 ≤ EI < 2.0), and 18 (45%) had high production (EI ≥ 2.0) (**Table 1**). The results demonstrate the cellulolytic potential of bacteria present in the Sisal rhizosphere. The isolate CR96 was the most promising, with an enzymatic index of 5.57.

Table 1 Enzymatic indices of cellulase-producing rhizobacteria associated with sisal. Means followed by the same letter do not significantly differ from each other according to the Scott–Knott test (p < 0.05).

Isolates	E.I.	Isolates	E.I.	Isolates	E.I.	Isolates	E.I.
CR01	2,92 ± 0,30 d	CR44	1,13 ± 0,11 f	CR72	1,12 ± 0,02 f	CR102	1,62 ± 0,64 f
CR04	4,26 ± 0,87 c	CR45	1,57 ± 0,10 f	CR73	1,07 ± 0,02 f	CR110	2,33 ± 1,46 e
CR10	4,12 ± 0,49 c	CR46	1,22 ± 0,06 f	CR92	2,03 ± 1,32 e	CR113	4,65 ± 1,33 b
CR11	3,93 ± 0,93 c	CR47	1,70 ± 0,14 f	CR93	1,58 ± 0,38 f	CR114	1,83 ± 0,74 e
CR12	3,69 ± 0,20 c	CR48	1,53 ± 0,42 f	CR94	1,01 ± 0,01 f	CR115	1,17 ± 0,03 f
CR21	3,17 ± 0,28 c	CR49	2,04 ± 0,33 e	CR95	3,96 ± 0,44 c	CR117	2,24 ± 0,70 e
CR22	2,70 ± 0,19 e	CR57	1,96 ± 0,26 e	CR96	5,57 ± 0,21 a	CR125	1,27 ± 0,08 f
CR37	4,34 ± 0,33 c	CR58	4,14 ± 0,27 c	CR97	1,45 ± 0,28 f	CR126	1,18 ± 0,07 f
CR39	1,28 ± 0,27 f	CR61	4,06 ± 0,32 c	CR99	1,44 ± 0,35 f	CR133	1,51 ± 0,34 f
CR40	1,54 ± 0,16 f	CR64	1,21 ± 0,11 f	CR100	1,17 ± 0,03 f	CR137	2,40 ± 0,82 e

Rhizosphere bacteria play an important role in the development of ecological niches through the production of bioactive compounds. Cellulases are directly linked to the carbon cycle through their bioconversion of cellulose into simple sugars that act in plant metabolism. When associated with phytohormones, these sugars can induce tolerance to abiotic stresses[13]. It is commonly assumed that fungi are the main bioconverters of plant biomass. However, the literature indicates that bacterial cellulases contribute significantly to the bioconversion process. These enzymes may have advantages compared to fungal enzymes due to the specific activity and stability of bacteria. The accelerated growth of bacteria and the higher production rate in a shorter time can represent an important factor in reducing enzyme production costs[14,15].

There are several bacterial strains that synthesize cellulases and tolerate extreme abiotic conditions, such as saline and dry environments and changes in soil pH. Studies have shown that cellulolytic bacteria such as *Pseudomonas* sp., *Bacillus* sp., and *Serratia* sp. [16,17] are tolerant to high temperatures and have become alternatives for the production of microbial enzymes for industrial use. The usefulness of microbial cellulases at the industrial scale is driven by the need for low-cost enzymes for the production of second-generation biofuels compared to conventional biomass pretreatment techniques

4 CONCLUSION

This study demonstrated high enzyme production by rhizobacteria associated with sisal, the bioenergetic potential of which plays a crucial role in the consolidation of new sources of renewable energy. The prospecting of microbial biocatalysts from sisal represents numerous advances in the development of sustainable processes and highlights the need for additional studies to strengthen new production models based on natural resources.

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ACKNOWLEDGEMENTS

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting the Master's scholarship to the first author, the Programa de Pós-Graduação em Biodiversidade Vegetal (PPGBVeg) and the Universidade do Estado da Bahia (UNEB).