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Bioprospecting Proteolytic Enzymes from Soil Actinobacteria of Brazilian Semiarid Zones

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Abstract
Actinobacteria from semiarid zones have been poorly studied and act as promising fonts of biotechnologically applicable hydrolytic enzymes. Proteases play a major role in industry, being responsible for the majority of the profit of this segment. Thus, this work focuses on the prospection of proteolytic enzymes from the semi-arid region of northeast Brazil, an understudied zone with buried biotechnological applications. Overall, 91,12% (41 of 42) of the studied strains were able to produce those enzymes, which shows a great initial potential for further studies.

Keywords: Protease, Actinomycetes, Enzymatic Potential.

1. Introduction

Enzymes are lytic proteins responsible for a wide range of metabolic functions that are essential to life maintenance. These biomolecules are specific in action due to two specialized peptidic zones: the binding site and the cleavage site. The binding site is responsible for the substrate attachment to the protein and the cleavage site has the ability to reduce the activation energy of the reaction, being able to effectively convert the substrates in products or the opposite, if possible. [1]

The aforementioned biocatalysts are grouped according to their catalytic mechanisms in six major classes: Oxirredutases, Transferases, Hydrolases, Lyases, Isomerases and Ligases [2]. Between these, the hydrolases group [EC-3] is described as enzymes that use water molecules to effectively cleave the substrate into products and deserves prominence in regard to its diffusion and unique importance in nature, once they are involved in the conversion of organic matter to oligomeric or monomeric subunits more easily used by cells [3].

Among the hydrolases, one of the most important enzymes from a biotechnological perspective are proteases or peptidases, enzymes that hydrolase the peptidic bounds between two aminoacid. Those enzymes are applicable in the beverage [4], cheese [5], meat [6], and cosmetics industries [7], among others, representing almost two thirds of all of the enzyme market yield [8], [a] sector that foresees to achieve $9.10 million of revenue by 2026 [9]. Therefore, continuous efforts are made to discover more reliable, stable, and profitable enzymes for industrial applications, where microbial enzymes play vital roles, as they fulfill all of the most desired industrial requirements: fast growth, high productivity, and easier downstream processes [10].

Actinobacteria are filamentous prokaryotic microorganisms that are deeply studied due to their extensive biotechnological applications given their vast number of produced biocompounds, like antibiotics [11] and enzymes [12], besides their ability to survive in harsh environments, as the case of northeastern Brazilian semiarid [13].

Therefore, the current study focuses on bioprospecting of extracellular proteolytic enzymes from actinobacterial sources isolated from soils from conservation units in the semi-arid Brazilian region, in order to identify promising biocatalysts for industry.

2. Materials and Methods

2.1 Actinobacteria

A total of 45 actinobacterial strains were selected from a pool of 347 isolates to perform this study. These strains were isolated from three different regions from the Brazilian semi-arid territory: 24 strains from Ecological Station of Aiuaba – CE (6º36'01" S 40º07'15" W; 6º44'35" S 40º19'19" W), 15 strains from National Park of Ubajara – CE (3°40'30" S 40°5' W; 3º49'30" S -40º52'30" W) and 6 strains from National Park of Sete Cidades – PI (04º02'08" S 41º40'45" W). The strains used in this study are deposited in the cultures collection of the Laboratory of Environmental Microbiology (LAMAB), located at the Biology Department of Federal University of Ceará (UFC).

2.2 Enzymatic Assay

To assess the capacity of each strain to produce proteases, a semi-quantitative method was performed utilizing the spot plate method. In this assay, a quadruplicate of spots of each actinobacteria strain was inoculated on Petri dishes containing the culture medium Skim Milk Agar (SMA): Skim Milk 5g, NaCl 3g, Peptone 3g and Agar 15g [14]. The dishes were incubated in the Biochemical Oxygen Demand Incubator (BOD) at 28±2ºC for 7 days. After the incubation stage, the plates were evaluated and a clear halo of hydrolysis around the colonies was considered as a positive test [14].

2.3 Enzymatic Index

The enzymatic index (EI) of protease production was individually calculated for each strain following the Eq. 1. Where Cd refers to the colony diameter in millimeters (mm) and Hd refers to the halo diameter also in mm [15]. For each strain, at least two assays were performed in order to increase the statistical reliability. At the end of the evaluation, the FEI (Final Enzymatic Index) was adopted as the mean between all of the individual EIs as well as the standard deviation (σ) of all EIs.

$$EI = Hd / Cd \quad (1)$$

2.4 Statistical analysis

The enzymatic indexes were then submitted to multivariate analysis in PAST v4.03.

3. Results & Discussion

Actinobacteria were already described as producers of a plethora of extracellular organic matter degrading hydrolytic proteins. These mainly saprophytes organisms are tasked to take care of the carbon recovery from biomass present in the environment [16], and their enzymatic apparatus is territorial dependent [17]. Although many efforts were already done in order to provide information about actinobacterial enzymatic production on Caatinga soils [12], only few data are available from protease producer actinobacteria and even less from Brazilian semiarid sources [18].

On account of that, the hydrolysis capacity of proteins from Brazilian northeastern semiarid actinobacteria was evaluated by using their enzymatic indices as the main parameter for analysis. In Figure 1 we can see both cases: a proteolytic enzyme producer strain (1.a) and a nonproducer (2.a), besides the Hd and Cd indicators to generate our IE measurement. All of the FEI and their respective standard deviations are described on Table 1 and a dendrogram coupled to a heatmap with clustering of the similar indices producers can be found on Figure 2.

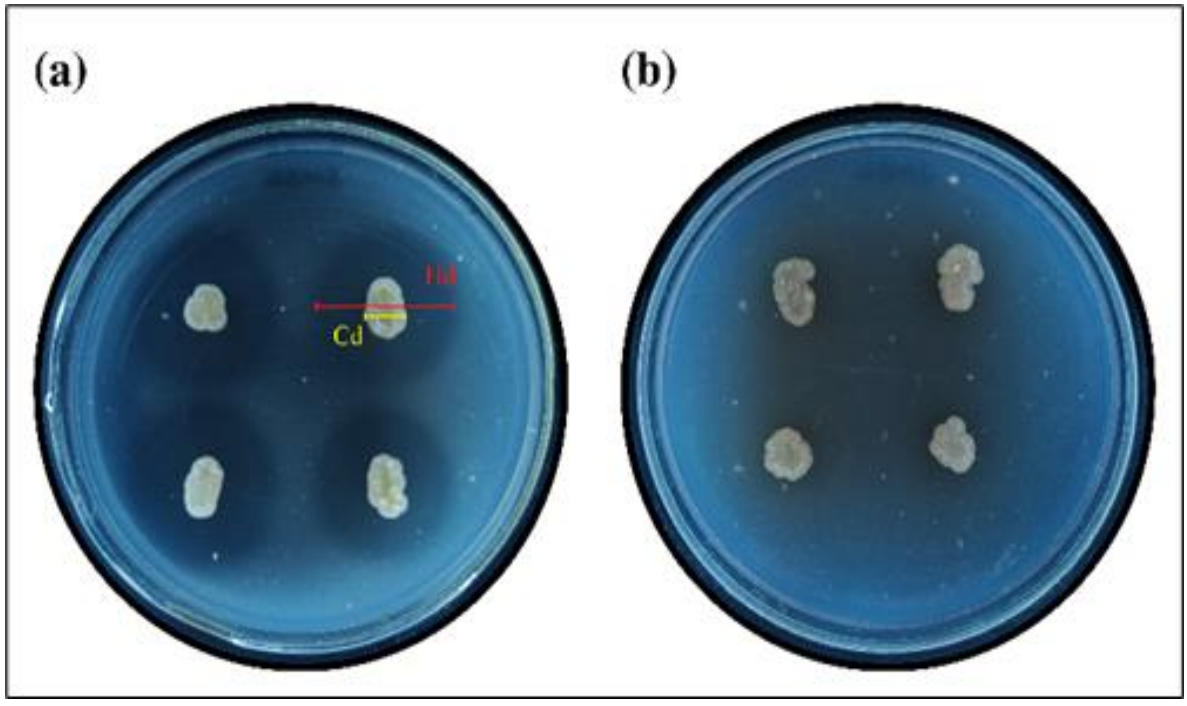

Fig. 1: Positive test for proteolytic assay, with indicators of Hd and Cd (a) Negative test for proteolytic assay (b).

From a total of 45 strains analyzed, a total of 41 (91.11%) were able to effectively produce degradation halos in SMA medium. As previously described, Caatinga-dweller actinobacteria were capable of producing proteolytic enzymes [18] as a mechanism for biomass degradation and antifungal activity [19]. Past studies suggest approximate proportion of proteolytic enzymes from actinobacteria of multiple environments [20,21]

Table 1: Actinobacteria strains Final Enzymatic Indexes (FEI) and their respectives standard deviations (σ).

Strain			Strain		
	FEI	σ		FEI	Σ
SN1	1.92	0.2	SN24	2.54	0.1
SN2	2.46	0.42	SN25	2.19	0.19
SN3	2.49	0.15	SN26	2.01	0.24
SN4	-	-	SN27	1.45	0.17
SN5	1.91	0.19	SN28	1.28	0.09
SN6	2.22	0.51	SN29	2.29	0.31
SN7	-	-	SN30	2.02	0.37
SN8	2.23	0.24	SN31	2.03	0.26
SN9	2.76	0.34	SN32	2.98	0.24
SN10	2.69	0.26	SN33	1.72	0.37
SN11	2.23	0.52	SN34	1.37	0.17
SN12	2.5	0.54	SN35	-	-
SN13	1.64	0.25	SN36	2.02	0.3
SN14	2.45	0.15	**SN37**	**3.19**	**0.16**
SN15	2.28	0.53	SN38	2.14	0.09
SN16	2.03	0.13	SN39	2.57	0.41
SN17	1.99	0.24	SN40	2.51	0.22
SN18	2.51	0.38	SN41	1.67	0.24
SN19	2.18	0.35	SN42	-	-
SN20	2.09	0.1	SN43	2.15	0.34
SN21	2.31	0.21	SN44	2.26	0.27
SN22	2.42	0.19	**SN45**	**1.35**	**0.15**
SN23	1.86	0.22	-	-	-
	Strain	FEI		Strain	FEI
Bigger FEI	SN32	3.19±0.16	**Lower FEI**	SN45	1.35±0.15

(-) Absence of hydrolysis halo. Font: authors.

Although all of the actinobacterial growth was performed under mesophilic conditions, previous works already reported that even though these semi-aridic prokaryotes act as meso-neutrophiles organisms at laboratory, they are able to support higher temperatures and salinitys if exposed to such conditions [18].

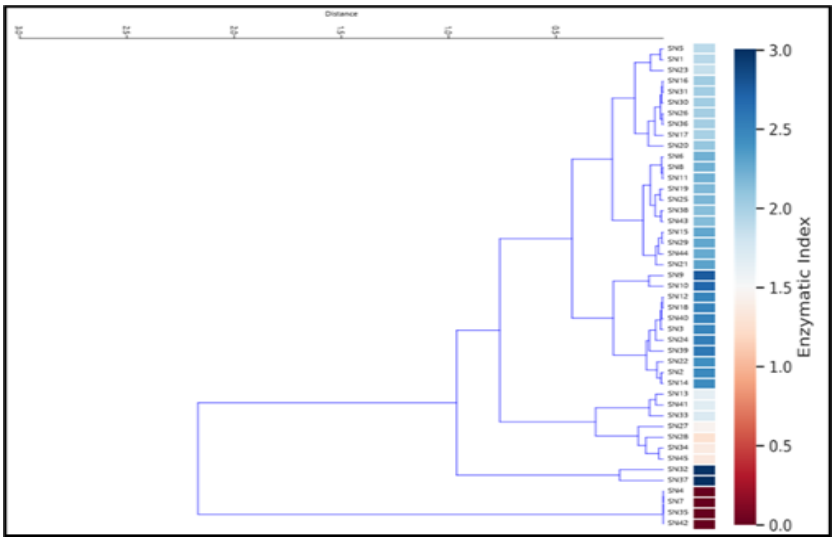

Fig. 2: Multivariate clustering analysis involving the enzymatic indexes of each strain coupled to a heatmap.

4. Conclusions

We conclude that actinobacterial strains from semi-arid Brazilian zones are able to produce, in high proportions and potentially thermoshalophilic, peptidases. Further studies on those strains are required to fulfill some gaps in this study: quantitative analysis of individual proteases, as well as pH, temperature, and salinity gradient tests.

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