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Production of Extracellular Pectinase from Cultivable Soil Bacteria Strains

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Abstract
Among the abundant and highly interactive microbial groups present in soil, actinobacteria stand out. An extracellular enzyme type produced by this phylum of bacteria is pectinases, which are enzymes capable of degrading pectin complex biomolecules. The present study aimed to evaluate the pectinolytic activity of actinobacteria. Forty-five strains of actinobacteria were selected according to their morphological characteristics, area where they were isolated and diversity of genera. The pectinolytic enzymatic index (EI) for each strain was determined by the ratio between the hydrolysis halo diameter (Dh) and the colony diameter (Dc). Of the 45 actinobacteria strains evaluated in this study, 35 (77.8%) showed pectinase production, the five most productive having enzymatic indices greater than 6. One thing to be noticed in our study is that more than 30% of or strains can be classified as strong producers of pectinase, which show the ecological and biotechnological potential of strains analyzed.

Keywords: Pectin, Extracellular enzymes; Actinobacteria; Ecological services.

1. Introduction
Soil constitutes heterogeneous environments, where physical, chemical and biological factors influence functional dynamics and affect the survival and community formation of microorganisms that inhabit this ecosystem [1]. Rhizosphere composes a unique environment in the soil and represents a favorable niche for several groups of bacteria, since root exudation makes available several substances that are used by microbial metabolism [2]. Among the abundant and highly interactive microbial groups present in soil, actinobacteria stand out.

Actinobacteria have strong biological activities, being prolific producers of secondary metabolites with high biological diversity in the soil. Bioactive molecules derived from actinobacteria represent approximately 70% of the naturally occurring compound currently in clinical use [3]. Within these numerous compounds produced by this bacterial group, pectinase is still little known and scientifically addressed, even though its industrial and ecological role has already been recognized [4].

Pectin is a high molecular weight polysaccharide commonly found in plants. Pectinase is a complex of enzymes involved in the biological degradation of pectin. They are important in plants for fruit ripening, cell signaling and adhesion. Pectinolytic enzymes have been classified as depolymerases, esterases, and protopectinases [5]. Market value of pectinases has been increased and it acquires the highest position among enzymes used commercially in industries and is estimated at almost 45$ billion. The ecological impact caused by the search for this enzyme is not yet well determined. It is known that the degradation of the plant cell wall is essential for the root nodulation of legumes, and pectinases are enzymes capable of breaking these structures. The pectinase-producing actinobacteria are able to degrade plant cell wall compounds, having their ecological role recognized by nutrient cycling [6].

Studies in semi-arid regions indicate the presence of actinobacteria in soils and the importance of this phylum in the establishment and maintenance of plant communities that

are crucial to the maintenance of ecosystem services [7]. Thus, the present study aimed to evaluate the pectinolytic activity of actinobacteria isolated from soils in the Brazilian semi-arid region.

Pectinases are classified according to the specific site of cleavage of pectic substances, divided into endo-polygalacturonases [EC-3.2.1.15], exo-polygalacturonases [EC-3.2.1.67], exo-polygalacturonosidases [EC-3.2.1.82]; rhamnogalacturonases [EC-3.2.1.-], endo-xylogalacturonan hydrolases [EC-3.2.1], endo-pectate lyases [EC-4.2.2.2], exo-pectate lyases [EC-4.2.2.9] and pectin lyases [EC-4.2.2.10] [8]. The scheme of reactions catalyzed by endo-polygalacturonases and their respective structural example can be found in Figure 1.

These enzymes are widely studied and produced, as they are highly demanded by a wide variety of industries. They are applicable in textile processing, reducing fermentation times, clarifying juices, reducing pulp viscosity, processing wines and extracting oils [9].

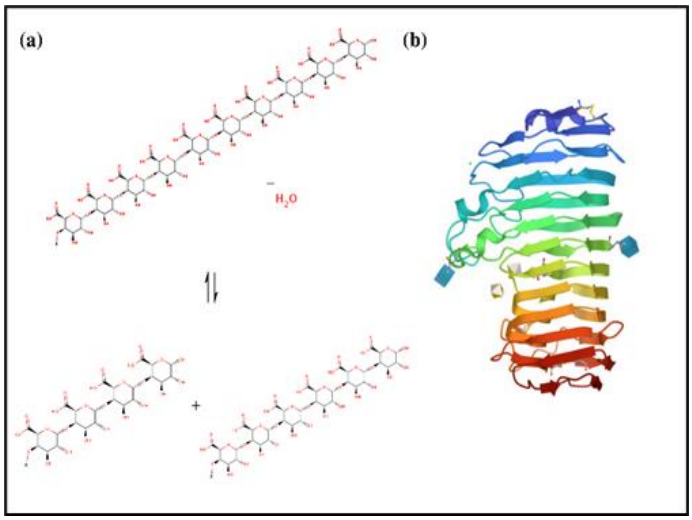

Fig. 1: a) Reaction scheme of endo-polygalacturonase-mediated pectin hydrolysis (b) Three-dimensional structure of endo-polygalacturonase isolated from Stereum purpureum. Source: Authors, 2022.

2. Materials and methods

2.1 Isolation of actinobacteria

The approach used for the isolation of microorganisms was the serial dilution combined with the spread plate method. Soil samples were collected in different areas of the Brazilian semi-arid region and 10g of each soil sample were diluted in 90mL of 0.85% saline solution. After the necessary procedures, 100µL of these solutions were spread onto Petri plates containing Casein-dextrose-agar (CDA) medium and incubated for a period of 7 days at 28±2 ºC. At the end of the incubation period, the number of actinobacteria colonies was counted for abundance calculations and the isolated colonies were inoculated individually in petri dishes containing CDA medium for subsequent procedures. A total of three tests were performed to improve the reliability of abundance calculations.

2.2 - Selection of bacterial strains

Bacterial strains were selected according to their morphological characteristics, area where they were isolated and diversity of genera. Forty-five strains of actinobacteria were selected for the *in vitro* experiment. The colonies were then grouped and individual nominations were designated by joining the abbreviation SN, referring to Northeastern Semiarid, to the number of each isolate (Table 1).

Table 1: Nomenclature of colonies according to area and location.

Location	***Strain identification***
UBJ	SN01
UBJ	SN02
UBJ	SN03, SN04, SN05, SN06, SN07, SN08
UBJ	SN09, SN10, SN12, SN13, SN20, SN32, SN33, SN34
UBJ	SN11, SN39
7CI	SN12
7CI	SN14
7CI	SN15
7CI	SN16
AIU	SN17, SN18, SN19
AIU	SN21, SN22
AIU	SN23, SN24, SN25
AIU	SN26, SN27, SN28, SN29
AIU	SN30, SN31
AIU	SN35, SN36, SN37, SN38
UBJ	SN40, SN41, SN42, SN43, SN44, SN45

UBJ = Ubajara 7CI = Sete Cidades AIU = Aiuaba (all locations in Brazil)

2.3 - Pectinolytic activity

Actinobacteria strains were inoculated in quadruplicate in the form of spots in Petri dishes containing TSA (Tryptic Soy Agar) medium, and incubated at 28ºC for 7 days in an B.O.D. incubator. At the end of this period, 10 ml of lugol was added to visualize the hydrolysis halo around the colonies [10].

2.4 - Determination of the enzymatic index (EI)

The enzymatic index of pectinolytic activity (EI) for each strain was determined by the diameter of the ratio between the hydrolysis halo (Dh) and the colony diameter (Dc) (Equation 1) measured in mm using a digital caliper. The arithmetic means of the EIs were evaluated using the Shapiro-Wilk normality test and, after the analysis of variance, the Tukey test was performed with 95% significance.

$$EI = Dh / Dc \quad (1)$$

3. Results & Discussion

Bacteria constitute diverse communities in soil and are considered as fundamental elements in the cycling of materials of different nature. Actinobacteria are part of a network of interactions responsible for the maintenance of important ecosystem services, such as the decomposition of polysaccharides [11]. In natural environments, polysaccharides usually bond and form complex structures that require the synergistic action of several enzymes for their complete degradation. Pectin is one of these enzymes, thus the presence of microorganisms that produce pectinase is important for an ecosystem balance [12].

The hydrolytic capacity of semiarid actinobacteria enzymes was evaluated by using their enzymatic indices as the main parameters for analysis. Figure 2 shows both cases: a pectinolytic enzyme producer strain (a) and a non-producer (b). Hd and Cd indicate the Halo and Colony diameters, respectively. Of the 45 actinobacteria strains evaluated in this study, 35 (77.8%) showed pectinase production, the five most productive having enzymatic indices greater than 6, which represents a high value (Table 2).

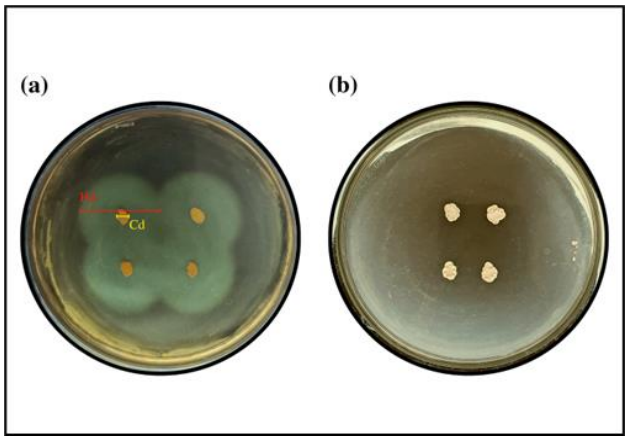

Fig. 2: Positive test for pectinase production (a) Negative test for pectinase production (b). Source: Authors, 2022.

Table 2: Pectinolytic enzymatic index of actinobacteria strains.

Strain	EI	σ	Strain	EI	σ
SN1	7,01	1,16	**SN24**	6,07	0,75
SN2	5,39	0,37	**SN25**	4,98	0,67
SN3	-	-	**SN26**	5,66	1,51
SN4	1,49	0,30	**SN27**	1,17	0,12
SN5	-	-	**SN28**	-	-
SN6	6,25	1,77	**SN29**	4,97	1,60
SN7	-	-	**SN30**	3,72	0,30
SN8	4,89	0,85	**SN31**	3,55	0,29
SN9	3,77	0,84	**SN32**	4,06	0,60
SN10	5,81	0,63	**SN33**	2,61	0,68
SN11	6,53	1,14	**SN34**	1,29	0,11
SN12	4,35	0,31	**SN35**	5,31	0,43
SN13	4,54	0,61	**SN36**	-	-
SN14	6,55	0,81	**SN37**	-	-
SN15	3,67	1,03	**SN38**	1,17	0,04
SN16	5,27	0,83	**SN39**	2,21	0,15
SN17	2,34	0,23	**SN40**	2,99	0,57
SN18	-	-	**SN41**	-	-
SN19	6,11	0,92	**SN42**	-	-
SN20	6,45	1,69	**SN43**	2,90	0,57
SN21	1,22	0,04	**SN44**	4,38	0,99
SN22	-	-	**SN45**	2,69	0,79
SN23	2,60	1,44			

EI= Enzymatic Index σ = standard deviation
Source: Authors, 2022.

Researchers from the Brazilian semi-arid region carried out a study about pectinase production by rhizobacteria [13]. They found out that 58% of the evaluated bacteria strains had pectinase production, which is a percentage lower than found out in this study. Another study carried out in the Brazilian Cerrado with bacteria isolated from the sugarcane rhizosphere demonstrated that of 36 strains, only 8% produced pectinolytic enzymes [14]. However, in the semi-arid region of the Mediterranean [15], the 13 rhizobia strains evaluated by the researchers' produced pectinases. This shows that even within the same group of bacteria, enzyme production is bound to many variables. This variation between the presence or absence of pectinases in strains identified in the different areas surveyed can be due to the presence of microbial genes that encode the formation of pectinases. One thing to be noticed in our study is that more than 30% of or strains can be classified as strong producers of pectinase, which show the ecological and biotechnological potential of strains analyzed (Figure 3).

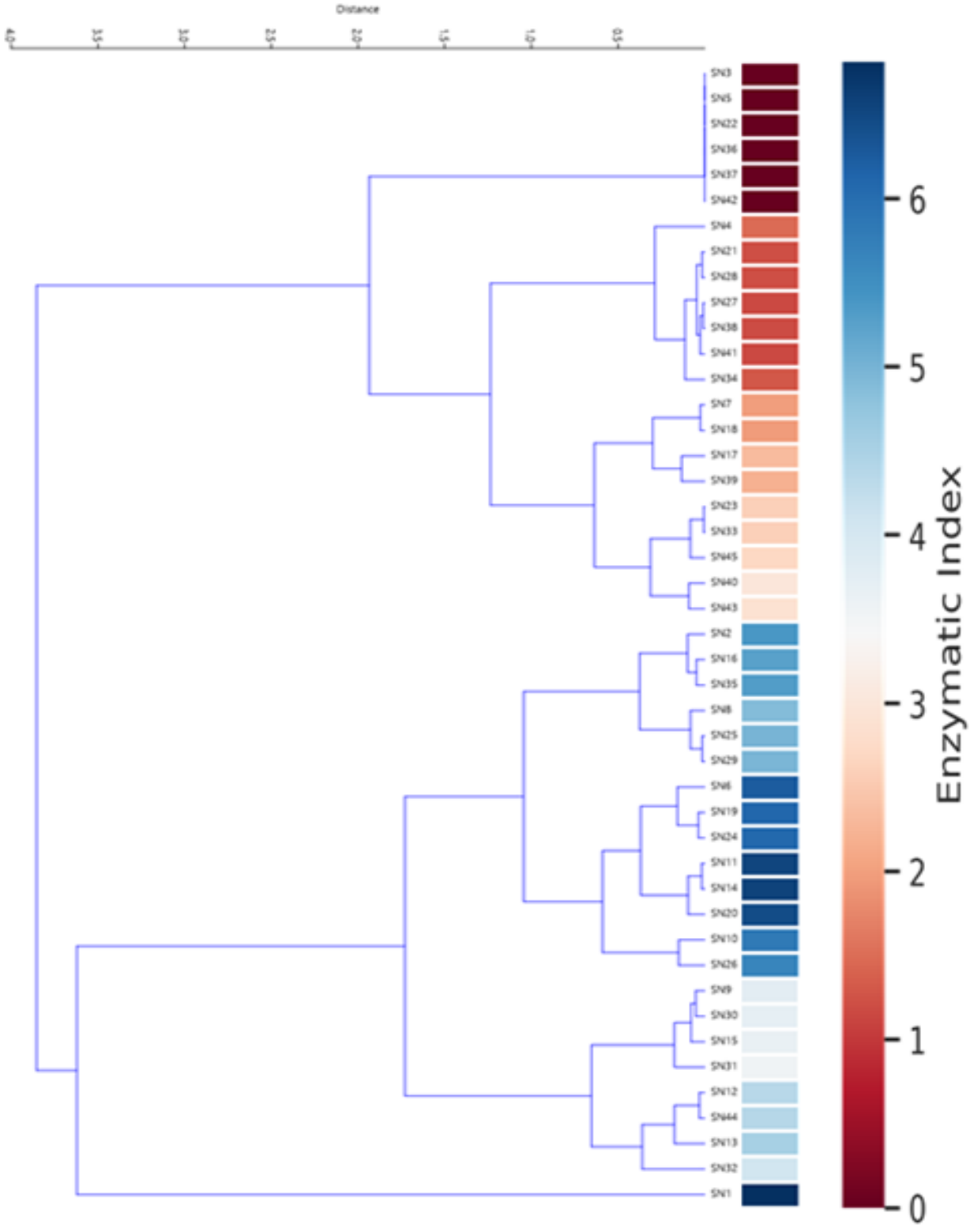

Fig. 3: Clustering analysis of enzymatic indexes of each strain coupled to a heatmap.
Source: Authors 2022

4. Conclusions

Actinobacteria strains isolated from semiarid Brazilian soils showed high pectinase enzymatic indexes. This phylum of bacteria has great ecological importance and this study reinforces the need for future analyses regarding enzyme production for this bacterial group. The images obtained, as well as the integration of the methodologies used, can help in future studies on this topic.

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