Revista ARACÊ
ISSN: 2358-2472

ISOLATION OF AMYLASE AND PROTEASE-PRODUCING BACTERIA FROM AGRO-INDUSTRIAL WASTE AND SOIL SAMPLES

https://doi.org/10.56238/arev7n8-083

Date of submission: 07/11/2025 **Date of publication:** 08/11/2025

Ryan Fernandes Vieira de Souza[1], Larissa Marques Negrett[2], Kauanny Wictoria Plenz[3], Rodrigo Matheus Pereira[4], Maricy Raquel Lindenbah Bonfá[5]

ABSTRACT

Agro-industrial and soil residues represent promising raw materials for biotechnological processes, contributing to a reduction in associated costs. These residues additionally serve as reservoirs of microorganisms, facilitating the bioprospecting of enzyme-producing bacteria of biotechnological significance. Soil environments, in particular, exhibit remarkable microbial biodiversity, with numerous microorganisms remaining uncharacterized. Proteases are extensively employed enzymes, commonly incorporated into detergents and widely utilized across the food, pharmaceutical, and textile sectors. Amylases are similarly indispensable in the food industry. Consequently, the present investigation focused on bioprospecting for bacteria demonstrating potential for the production of these enzymes from two distinct sample origins: oily residue generated from soybean processing, supplied by the COAMO cooperative (Dourados-MS unit), and soil collected from the Várzeas do Rio Ivinhema State Park. To this end, two differential culture media were formulated, each augmented with specific substrates to induce the respective production of protease and amylase enzymes. Following the isolation of pure colonies, a qualitative assessment of enzymatic activity was performed. The Enzyme Index (EI) was determined via the cup-plate method, calculated as the ratio of the degradation halo diameter to the colony diameter. Isolates presenting an EI exceeding 2.0 mm were designated as robust enzyme producers. Subsequently, morphotintorial classification was executed utilizing the Gram staining method on the colonies identified as strong enzyme producers. The outcomes revealed the successful isolation of one bacterium exhibiting protease production potential and two bacteria with amylase production potential from the agro-industrial waste sample. Conversely, the soil sample yielded one bacterium with amylase production potential and five with protease production potential. All isolated strains in this study were characterized as Gram-positive rods and tested positive for catalase Productions.

[1] Master's student of the Interunit Postgraduate Program in Bioinformatics. Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais (ICB, UFMG).
E-mail: ryanfernandes44@gmail.com ORCID: https://orcid.org/0009-0006-1393-7224
[2] Undergraduate student in Bachelor of Biotechnology. Faculdade de Ciências Biológicas e Ambientais da Universidade Federal da Grande Dourados (FCBA, UFGD).
E-mail: marquesnegrettlarissa@gmail.com ORCID: https://orcid.org/0009-0000-9617-4889
[3] Undergraduate student in Bachelor of Biotechnology. Faculdade de Ciências Biológicas e Ambientais da Universidade Federal da Grande Dourados (FCBA, UFGD). E-mail: kakawictoria8@gmail.com
ORCID: https://orcid.org/0009-0005-8399-2803
[4] PhD in Agricultural Microbiology. Programa de Pós-Graduação em Microbiologia Agropecuária da Universidade Estadual Paulista "Júlio de Mesquita Filho" (PPG-MICRO, UNESP).
E-mail: rodrigopereira@ufgd.edu.br ORCID: https://orcid.org/0000-0001-6025-5118
[5] PhD in Food Science. Programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Campinas (PPGCA, UNICAMP). E-mail: maricybonfa@ufgd.edu.br
ORCID: https://orcid.org/0000-0002-6978-4769

Revista ARACÊ
ISSN: 2358-2472

Keywords: Biotechnological Potential. Enzymes. Bioprospecting.

ISOLAMENTO DE BACTÉRIAS COM POTENCIAL DE PRODUÇÃO DE AMILASE E PROTEASE A PARTIR DE AMOSTRA DE RESÍDUO AGROINDUSTRIAL E DE SOLO

RESUMO
Os resíduos agroindustriais e de solo são potenciais matérias-primas para os processos biotecnológicos, e tendem a reduzir os custos destes processos. Além disso, microrganismos podem ser encontrados nestes resíduos, e a depender de sua composição, é possível bioprospectar bactérias produtoras de enzimas com de interesse biotecnológico. O solo também é um ambiente rico em biodiversidade microbiana, sendo que muitos microrganismos nem sequer foram estudados. Uma das enzimas mais utilizadas e produtos são as proteases, geralmente presentes em sabões e detergentes, na indústria alimentícia, farmacêutica e têxtil. As amilases são também aplicadas na indústria de alimentos. Dessa forma, o presente estudo bioprospectou bactérias com potencial produtor das enzimas citadas em dois diferentes tipos de amostras: resíduo de borra oleosa da produção de soja, cedido pela cooperativa COAMO, unidade de Dourados-MS, e solo coletado no Parque Estadual das Várzeas do Rio Ivinhema. Para isso, foram preparados dois meios de cultivo diferentes, cada um suplementado com substratos específicos para induzir a produção das enzimas protease e amilase. Após a obtenção de colônias puras, foi realizada uma avaliação qualitativa da produção das enzimas. O método utilizado foi o *cup plate*, determinando o Índice Enzimático (IE) pela relação entre o diâmetro do halo de degradação do meio de cultivo e o diâmetro da colônia. Isolados com IE superiores a 2,0 mm foram considerados bons produtores enzimáticos. Por fim, foi realizada a classificação morfotintorial pelo método de coloração de Gram das colônias avaliadas como boas produtoras enzimáticas. Como resultado, a partir da amostra de Agro-industrial waste, foi isolada uma bactéria com potencial de produção de protease e duas bactérias com potencial de produção de amilase. Já a partir da Soil sample, foi isolada uma bactéria com potencial de produção de amilase e cinco com potencial de produção de protease. Todas as cepas isoladas no presente estudo foram classificadas como bastonetes Gram-positivos e positivas para produção de catalase.

Palavras-chave: Potencial Biotecnológico. Enzimas. Bioprospecção.

AISLAMIENTO DE BACTERIAS CON POTENCIAL PARA PRODUCIR AMILASA Y PROTEASA A PARTIR DE MUESTRAS DE RESIDUOS AGROINDUSTRIALES Y DEL SUELO

RESUMEN
Los residuos agroindustriales y del suelo representan materias primas prometedoras para los procesos biotecnológicos, contribuyendo a la reducción de los costos asociados. Estos residuos, además, sirven como reservorios de microorganismos, facilitando la bioprospección de bacterias productoras de enzimas de importancia biotecnológica. Los ambientes edáficos, en particular, exhiben una notable biodiversidad microbiana, con numerosos microorganismos sin caracterizar. Las proteasas son enzimas ampliamente utilizadas, comúnmente incorporadas en detergentes y ampliamente utilizadas en los sectores alimentario, farmacéutico y textil. Las amilasas son igualmente indispensables en la industria alimentaria. Por consiguiente, la presente investigación se centró en la

Revista ARACÊ
ISSN: 2358-2472

bioprospección de bacterias que demuestran potencial para la producción de estas enzimas a partir de dos orígenes de muestra distintos: residuos oleosos generados por el procesamiento de soja, suministrados por la cooperativa COAMO (unidad Dourados-MS), y suelo recolectado del Parque Estatal Várzeas do Rio Ivinhema. Para ello, se formularon dos medios de cultivo diferenciales, cada uno enriquecido con sustratos específicos para inducir la producción respectiva de proteasas y amilasas. Tras el aislamiento de colonias puras, se realizó una evaluación cualitativa de la actividad enzimática. El Índice Enzimático (IE) se determinó mediante el método de copa-placa, calculado como la relación entre el diámetro del halo de degradación y el diámetro de la colonia. Los aislados con un IE superior a 2,0 mm se clasificaron como productores enzimáticos robustos. Posteriormente, se realizó una clasificación morfotintorial mediante tinción de Gram en las colonias identificadas como productoras enzimáticas robustas. Los resultados revelaron el aislamiento exitoso de una bacteria con potencial para la producción de proteasas y dos bacterias con potencial para la producción de amilasas de la muestra de residuos agroindustriales. Por otro lado, la muestra de suelo arrojó una bacteria con potencial para la producción de amilasas y cinco con potencial para la producción de proteasas. Todas las cepas aisladas en este estudio se caracterizaron como bacilos grampositivos y dieron positivo para la producción de catalasa.

Palabras clave: Potencial Biotecnológico. Enzimas. Bioprospección.

Revista ARACÊ
ISSN: 2358-2472

1 INTRODUCTION

Globally, the escalating volume of industrially generated waste constitutes a severe environmental concern. Particularly in Brazil, with its predominant agro-industrial sector, the strategic utilization of this waste as feedstock for biotechnological product development offers a compelling solution. This initiative effectively mitigates the increasing apprehension concerning waste disposal while simultaneously offering concrete remedies for the environmental repercussions linked to these by-products domestically (Vivek et al., 2022)

In addition to agro-industrial waste, soil is recognized as a strategic environment in the pursuit of microorganisms exhibiting biotechnological potential. Being a highly dynamic and diverse ecosystem, it hosts bacteria equipped with distinct metabolic mechanisms for thriving under diverse environmental pressures, numerous of which are amenable to industrial exploitation (Razzaq et al., 2019).

Bioprospecting for microorganisms in these environments has proven an effective strategy for identifying novel sources with biotechnological potential. For instance, fungal isolates obtained through bioprospecting in a regional agroindustry in Rio de Janeiro, Brazil, were found to be producers of pectinases with promising biotechnological applications (Junior et al., 2021). This approach not only helps valorize agro-industrial waste, thereby reducing its environmental footprint, but also promotes the discovery of new microorganisms capable of producing enzymes of economic interest. Furthermore, these processes contribute significantly to sustainability and innovation within the biotechnology sector (Han et al., 2019).

The use of these enzymes ensures the efficiency of natural processes, in addition to reducing or eliminating environmental risks associated with waste or effluents resulting from industrial production. They are also capable of performing important functions, such as the biodegradation of toxic compounds, effluent treatment, and environmental bioremediation (Alves, Paiva, 2018; Espínola, GorlachLira, 2018; Oliveira, Campos, 2020).

Among the various enzymes of economic interest, amylases and proteases stand out due to their characteristics that are highly applicable in the industrial sector. The former have the ability to hydrolyze starch and can be applied mainly in the food industry, beverage production, and the textile industry. Proteases cleave peptide bonds and are also applicable in the food industry and detergent production (Alves, Paiva, 2018; Bernal, 2019; Espínola, GorlachLira, 2018; Oliveira, Campos, 2020). Thus, the present study aimed to isolate bacteria with the potential to produce the aforementioned enzymes in samples of waste from

Revista ARACÊ
ISSN: 2358-2472

the COAMO agroindustry unit in Dourados, MS, and in soil samples collected in the Várzeas do Rio Ivinhema State Park.

2 METHODOLOGY

2.1 SAMPLE COLLECTION AND BACTERIA ISOLATION

The sample of agroindustrial residue from soybean oil production was provided by the COAMO cooperative, Dourados-MS unit. The soil sample was collected at the Várzeas do Rio Ivinhema State Park. Each type of sample consisted of three subsamples (≈ 3.33 g each) and was subsequently homogenized for bioprospecting.

From the homogenized sample of agroindustrial residue, 10 g were separated, to which 88 mL of sterile saline solution (0.9% NaCl) and 2.0 mL of Tween 80, also sterilized, were added in Erlenmeyer flasks. The flasks were shaken in an orbital shaker at 300 rpm for 30 minutes. The same procedure was applied to the soil sample, except for the addition of Tween 80, and the total volume was adjusted to 90 mL with saline solution (0.9% NaCl).

From this, serial dilutions of the suspension of the two types of samples were made, consisting of 100 µL inoculum of dilutions 10-4 and 10-5 by the surface spreading method in Petri dishes in two different ISP9 adapted culture media (Matias et al., 2009), one supplemented with skim milk (2% w/v) and the other with soluble starch (2% w/v), with macronutrient concentrations of: (2.64 g.L-1) (NH4)2SO4; (5.65 g.L-1) K2HPO4.3H2O and (2.38 g.L-1) KH2PO4; and micronutrient concentrations: (0.6 g.L-1) CuSO4.5H2O; (0.1 g.L-1) FeSO4.7H2O; (0.79 g.L-1) MnSO4.H2O; (0.15 g.L-1) ZnSO4.7H2O and to solidify the medium, 20 g. L-1 bacterial agar was used to solidify the medium. The pH of each medium was adjusted to 7.0 before adding the agar, and once ready, the medium was autoclaved at 121°C for 20 minutes. After autoclaving, 3 mL of fluconazole 0.01 g.mL-1 was added to each liter of medium to prevent fungal growth. The plates were incubated for 48 hours in a BOD incubator at a temperature of 30°C. After growth, bacteria that presented a degradation halo around the colony were selected. The bacteria were streaked by depletion on new plates to obtain pure colonies.

2.2 ASSESSMENT OF ENZYMATIC POTENTIAL

The enzymatic potential of the isolated bacteria was evaluated using the cup plate method (Dingles et al., 1953). Using a sterile loop, an isolated colony was applied to four quadrants of a Petri dish containing the specific solidified medium. This is done so that the

Revista ARACÊ
ISSN: 2358-2472

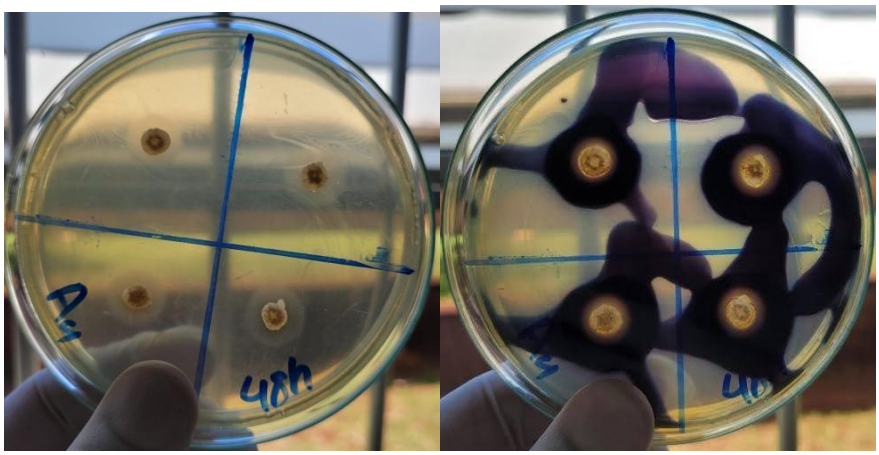
colony grows in only one specific spot on the plate and away from the others. After that, the plates were incubated in BOD at 30 °C for 24 and 48 hours. Then, the plates were covered with Lugol's solution (2.0 g KI and 1.0 g iodine in 300 ml of water) for 10 seconds until the degradation halos around the colonies became evident. Enzymatic potential was determined by calculating the Enzyme Index (EI), which measures the ratio between the average diameter of the degradation halo and the average diameter of the colony.

2.3 CLASSIFICATION OF ISOLATED BACTERIA

The bacteria selected as good enzyme producers (IE greater than 2 mm) were again cultured in Plate Count Agar (PCA, according to the manufacturer's instructions) and morphotintorial classification was performed (Figure 1). For this purpose, colonies with a maximum growth of 48 hours were collected for the preparation of slides stained by Gram staining. First, a smear (from each colony) was made on a slide and crystal violet was applied for 30 seconds; then it was stained with Lugol's solution for 60 seconds; then washed with a bleaching solution composed of ethanol and acetone for approximately 15 seconds; then washed again with distilled water; then fuchsin was applied and left to act for 30 seconds; the slide was washed again with distilled water and placed under a microscope at 1000x magnification to view the cells. Colonies that appeared pink or reddish were initially considered Gram-negative bacteria, and those that appeared purple or bluish were considered Gram-positive.

Figure 1

Isolated colonies covered with Lugol's solution (2.0 g KI and 1.0 g iodine in 300 ml water).



Revista ARACÊ
ISSN: 2358-2472

To confirm the Gram stain result, a KOH test was performed, in which a sample of the isolated colony was placed on a glass slide using a platinum loop. Two drops of 3% KOH solution were added, and the material was mixed for approximately 30 seconds with circular movements. During stirring, the movements of the sample with the loop were observed to identify the presence of viscosity in the sample. In the case of Gram-negative bacteria, KOH breaks down the cell wall, exposing the DNA, resulting in visible viscosity. If the bacteria are misclassified as Gram-negative, the absence of viscosity would indicate that the correct classification is Gram-positive, whose cell wall is not affected by the 3% KOH solution.

For the catalase test, a platinum loop was used to collect a substantial amount of the target bacteria, followed by smearing on a slide. Next, a drop of 3% hydrogen peroxide was applied to the bacteria on the slide. The immediate appearance of bubbles indicating effervescence was considered a positive result, demonstrating the conversion of H2O2 into water and oxygen gas. In contrast, the absence of activity, such as bubble formation or effervescence, was interpreted as a negative result. It is important to note that this test was performed on bacteria previously identified as Gram-positive.

3 RESULTS

3.1 ISOLATION OF BACTERIA

From the agroindustrial waste sample provided by the COAMO cooperative, Dourados-MS unit, 36 bacterial colonies were isolated, 18 of which were cultivated in a medium supplemented with soluble starch (2% w/v) and 18 in a medium supplemented with skim milk (2% w/v). From the soil sample collected at the Várzeas do Rio Ivinhema State Park, 30 bacterial colonies were obtained, of which 12 were isolated in medium supplemented with soluble starch (2% w/v) and 18 were isolated in medium supplemented with skim milk (2% w/v). The isolation of the colonies was performed by serial dilution, followed by depletion in Petri dishes (Table 1).

Revista ARACÊ
ISSN: 2358-2472

Table 1

Identification of isolated colonies and their respective origins.

Isolads	Sample type
A1	Agro-industrial waste
A2	Agro-industrial waste
A3	Agro-industrial waste
A4	Agro-industrial waste
A5	Agro-industrial waste
A6	Agro-industrial waste
A7	Agro-industrial waste
A8	Agro-industrial waste
A9	Agro-industrial waste
A10	Agro-industrial waste
A11	Agro-industrial waste
A12	Agro-industrial waste
A13	Agro-industrial waste
A14	Agro-industrial waste
A15	Agro-industrial waste
A16	Agro-industrial waste
A17	Agro-industrial waste
A18	Agro-industrial waste
P1	Agro-industrial waste
P2	Agro-industrial waste
P3	Agro-industrial waste
P4	Agro-industrial waste
P5	Agro-industrial waste
P6	Agro-industrial waste
P7	Agro-industrial waste
P8	Agro-industrial waste
P9	Agro-industrial waste
P10	Agro-industrial waste
P11	Agro-industrial waste
P12	Agro-industrial waste
P13	Agro-industrial waste
P14	Agro-industrial waste
P15	Agro-industrial waste
P16	Agro-industrial waste
P17	Agro-industrial waste
P18	Agro-industrial waste
Ami 1	Soil sample
Ami 2	Soil sample
Ami 3	Soil sample
Ami 4	Soil sample
Ami 5	Soil sample
Ami 6	Soil sample
Ami 7	Soil sample
Ami 8	Soil sample
Ami 9	Soil sample
Ami 10	Soil sample
Ami 11	Soil sample
Ami 12	Soil sample
Prot 1	Soil sample
Prot 2	Soil sample
Prot 3	Soil sample
Prot 4	Soil sample
Prot 5	Soil sample
Prot 6	Soil sample
Prot 7	Soil sample

Revista ARACÊ
ISSN: 2358-2472

Prot 8	Soil sample
Prot 9	Soil sample
Prot 10	Soil sample
Prot 11	Soil sample
Prot 12	Soil sample
Prot 13	Soil sample
Prot 14	Soil sample
Prot 15	Soil sample
Prot 16	Soil sample
Prot 17	Soil sample
Prot 18	Soil sample

The enzymatic potential of each strain was determined using the Enzyme Index (EI), with values equal to or greater than 2.0 (EI ≥ 2.0) being considered significant, indicating a strong potential for the production of the enzymes of interest, amylase and protease. Each strain was replicated in quadruplicate and incubated in a BOD oven at 30 °C for 24 and 48 hours. After this period, the diameters of the colonies and degradation halos were measured in each replica. The average of these values was then calculated to obtain the EI, as shown in Table 2.

Table 2

Enzyme index of isolates at 24 and 48 hours.

	Enzyme index			
	24 hours		48 hours	
Isolads	x̄	σ	x̄	σ
A1	1,15	0,19	1,17	1,1
A2	1,37	0,11	2,0	0,12
A3	1,31	0,23	1,35	0,09
A4	ND	ND	1,64	1,14
A5	1,38	0,22	1,03	0,035
A6	2,62	0,22	2,03	0,1
A7	ND	ND	ND	ND
A8	1,17	0,07	1,05	0,013
A9	1,21	0,07	1,20	0,055
A10	1,10	0,01	1,20	0,01
A11	ND	ND	ND	ND
A12	ND	ND	ND	ND
A13	ND	ND	1,27	0,13
A14	1,24	0,02	ND	ND
A15	1,13	0,007	1,0	0,2
A16	2,25	0.25	2,00	0,07
A17	ND	ND	ND	ND
A18	1,2	0,01	1,21	0,06
P1	ND	ND	ND	ND
P2	ND	ND	ND	ND
P3	1,67	0,07	2,0	0,19
P4	1.75	0,025	1,16	0,015
P5	ND	ND	ND	ND
P6	ND	ND	ND	ND
P7	ND	ND	ND	ND

Revista ARACÊ
ISSN: 2358-2472

P8	ND	ND	ND	ND
P9	1,24	0,095	1,5	0,07
P10	1,4	0,04	1,78	0,015
P11	ND	ND	ND	ND
P12	2,22	0.25	2,14	0,06
P13	ND	ND	ND	ND
P14	ND	ND	ND	ND
P15	ND	ND	ND	ND
P16	1,25	0,025	1,82	0,011
P17	1,83	0,17	1,23	0,045
P18	1,56	0,125	1,4	0,10
Ami 1	ND	ND	ND	ND
Ami 2	ND	ND	ND	ND
Ami 3	ND	ND	1,63	0,321
Ami 4	ND	ND	ND	ND
Ami 5	ND	ND	ND	ND
Ami 6	ND	ND	ND	ND
Ami 7	ND	ND	ND	ND
Ami 8	ND	ND	ND	ND
Ami 9	ND	ND	ND	ND
Ami 10	ND	ND	ND	ND
Ami 11	ND	ND	ND	ND
Ami 12	ND	ND	ND	ND
Prot 1	ND	ND	ND	ND
Prot 2	ND	ND	ND	ND
Prot 3	1,50	0,43	1,80	0,360
Prot 4	2,00	0,1	2,20	0,264
Prot 5	1,73	0,230	1,80	0,1
Prot 6	ND	ND	ND	ND
Prot 7	ND	ND	ND	ND
Prot 8	1,20	0,2	1,46	0,305
Prot 9	1,23	0,152	1,60	0,264
Prot 10	2,60	0,2	3,03	0,585
Prot 11	ND	ND	ND	ND
Prot 12	1,16	0,152	1,43	0,208
Prot 13	2,10	0,346	2,60	0,264
Prot 14	2,46	0,416	2,95	0,35
Prot 15	ND	ND	ND	ND
Prot 16	1,70	0,458	1,10	0,458
Prot 17	2,16	0,115	2,63	0,288
Prot 18	ND	ND	ND	ND

ND – Undetermined. x̄ - Average. σ – Standard deviation.

Revista ARACÊ
ISSN: 2358-2472

The assessment of enzymatic potential, performed using the cup plate method, revealed that, among the colonies isolated in minimal medium supplemented with soluble starch from the agroindustrial sample, two (A6 and A16) had an average Enzyme Index (EI) greater than 2.0 mm in the 24- and 48-hour periods. Another colony, Ami3, isolated from soil, had an EI greater than 2.0 mm only in the 48-hour period. Among the colonies isolated in minimal medium supplemented with skim milk, colony P17, from the agroindustrial sample, had an average EI greater than 2.0 mm in both periods analyzed. On the other hand, among the colonies isolated from the soil sample, six (P17, Prot 4, Prot 10, Prot 13, Prot 14, and Prot 17) exhibited an average IE greater than 2.0 mm in the same periods (Table 3). The other colonies isolated by the initial screening did not show visible degradation halos or had a diameter of less than 2.0 mm.

Table 3

Enzyme indices of isolates potentially producing amylase and protease after 24 and 48 hours of incubation at 30 °C.

	Enzyme index	
Isolads	24 hours	48 hours
A6	2,62	2,03
A16	2,25	2,00
Ami 3	ND	2,00
P17	2,22	2,14
Prot 4	2,01	2,80
Prot 10	2,80	3,70
Prot 13	1,70	2,30
Prot 14	1,80	2,10
Prot 17	2,10	2,70

ND – Undetermined.

In addition, all isolates were classified as bacteria. The morphology of the nine bacteria mentioned was confirmed and characterized as Gram-positive through Gram staining, and the KOH test was confirmatory, ensuring the results for the morphology of the isolates. Next, the catalase test was performed for the isolates confirmed as Gram-positive according to the results described in Table 4.

Revista ARACÊ
ISSN: 2358-2472

Table 4

Morphological classification of isolates potentially producing amylase and protease.

Isolads	Gram	Morphology	KOH	Catalase
A6	+	Bastonetes	NR	+
A16	+	Bastonetes	NR	+
Ami 3	+	Bastonetes	NR	+
P17	+	Bastonetes	NR	+
Prot 4	+	Bastonetes	NR	+
Prot 10	+	Bastonetes	NR	+
Prot 13	+	Bastonetes	NR	+
Prot 14	+	Bastonetes	NR	+
Prot 17	+	Bastonetes	NR	+

+ - Positive; NR – Non-reactive.

Based on the Gram stain test, it was found that the isolates from both soil and agroindustrial samples are bacteria and showed purple staining. When analyzing their morphologies under 1000x magnification, it became evident that they were rods, possibly from the Bacillaceae family. In addition, the KOH test confirmed the previous classification as Gram-positive, since no viscosity was observed in the samples when a 3% KOH solution was applied. They are also positive for catalase, as bubbles quickly formed when 3% hydrogen peroxide was applied.

4 DISCUSSION

The morphotintorial characteristics and catalase production include the bacteria isolated in the present study to the Bacillaceae family. The Bacillaceae family, composed of Gram-positive aerobic or facultative anaerobic bacteria, is recognized as a group of microorganisms of great relevance in the production of enzymes with applications in various sectors. Many scientific studies prove the importance of the Bacillaceae family in this context, highlighting its ability to synthesize enzymes with distinct properties and functions.

An example of this is the recent study published by Silva et al. (2023), which investigated the production of amylases by different species of Bacillaceae. The study showed that the species Bacillus subtilis had the highest amylase activity, indicating its potential for industrial application in the production of biofuels and in the food industry.

Revista ARACÊ
ISSN: 2358-2472

Another relevant study, published in 2022 in the journal “Biotechnology Reports” by Oliveira et al (2022), evaluated the production of proteases by Bacillus cereus. The results demonstrated that the strain studied exhibited high proteolytic activity, with potential for application in the leather and detergent industries.

In the study by Oliveira & Campos (2020), compost samples were used to isolate bacteria that produce enzymes of commercial interest. The results indicated the identification of four strains potentially producing proteases, with the highest Enzyme Index (EI) recorded being 2.14. Comparatively, the strains isolated from the agro-industrial waste sample in the present study showed similar or higher potential, with EI ranging from 2.00 to 2.62. Among the strains isolated from the soil, five showed similar results, with values ranging from 1.70 to 3.50 cm. In the same study, five potentially amylase-producing strains were isolated, with EI between 1.13 and 2.15, values similar to those obtained for isolates in medium supplemented with soluble starch, both from agro-industrial waste samples and soil samples.

In the study conducted by Dhayalan et al. (2022), bacteria with the potential to produce the protease enzyme were isolated from the intestines of Systomus sarana fish, measured qualitatively by the enzyme index. Of the 11 strains obtained, the average enzyme index was close to 4.00 mm. The bacterium B. thuringiensis, identified as strain SS5, stood out with an index of 6.83 mm. In another study conducted by Mohamed et al. (2023), three isolates of the genus Bacillus from soil were tested, which presented enzyme indices ranging from 1.7 to 2.5 mm. These two studies presented results similar to the EI obtained from the isolates in Skim Milk-supplemented medium in the present study.

The Bacillus genus is recognized as the main producer of proteases, capable of generating large amounts of neutral and alkaline proteolytic enzymes. These enzymes have remarkable characteristics, including high stability under extreme conditions of temperature, pH, presence of organic solvents, detergents, and oxidizing compounds, as highlighted by Contesini et al. (2018). In addition, it has been reported that members of this genus are capable of producing at least ten distinct extracellular enzymes. It is also known that 32 of the 48 Bacillus species listed by Buchanan & Gibbons (1974) are capable of degrading starch. Therefore, the genus Bacillus has great potential to dominate the enzyme sector as a source of proteases and amylases.

These studies, together with other scientific papers published in renowned journals, prove the importance of the Bacillaceae family in the production of enzymes with various industrial and biotechnological applications. The ability to synthesize enzymes with specific

Revista ARACÊ
ISSN: 2358-2472

properties, combined with the ease of cultivation and robustness of bacteria in this family, makes them valuable tools for the development of new products and processes.

In this way, the results of the present study were promising, indicating the presence of microorganisms found in samples of agro-industrial waste and soil with significant potential for biotechnological and industrial applications. The screening method used, based on measuring degradation halos around the colonies, proved effective in identifying enzyme-producing strains. This research not only expands knowledge about the microbial biodiversity of agro-industrial residues but also paves the way for future studies focused on utilizing such waste to obtain microorganisms with the potential to produce enzymes applicable in biotechnological and industrial processes. This is essential for the development of sustainable and innovative solutions in areas such as the food industry, agriculture, and waste treatment.

5 CONCLUSION

From the agro-industrial sample, two Gram-positive bacteria exhibiting amylase production potential and one with protease production potential were successfully isolated. Conversely, the soil sample yielded one bacterium with amylase production potential and six with protease production potential. All nine isolated strains were characterized as Gram-positive and rod-shaped, and their catalase production suggests a probable affiliation with the Bacillaceae family.

Qualitative assessment using the enzyme index (EI) method (cup plate) revealed that these isolates displayed EI values exceeding 2 mm, indicating their significant potential as enzyme producers. Moreover, the EIs obtained align with those previously documented for other Bacillus isolates recognized for their production of the aforementioned enzymes.

Consequently, this study establishes that waste from the COAMO agro-industry (Dourados unit, MS) and soil from the Ivinhema River Floodplain State Park serve as valuable sources of bacteria possessing biotechnological potential for the production of commercially important enzymes such as proteases and amylases.

ACKNOWLEDGMENTS

I would like to thank the Foundation for the Support of the Development of Education, Science, and Technology of the State of Mato Grosso do Sul (Fundect) for the financial

Revista ARACÊ
ISSN: 2358-2472

support and encouragement of scientific research, as well as the Federal University of Grande Dourados (UFGD).

REFERENCES

Alves, B. A.; Paiva, A. D. Bioprospecção de bactérias produtoras de enzimas de interesse biotecnológico. v. 3, pag 1–13, Jul, 2018.

Bernal, S. P. F. Instituto latino-americano de ciências da vida e da natureza programa de pós-graduação em biociências. Jan, 2019.

Buchanan, R. E.; Gibbons, N. E.Bergey',s. *Manual oj' Determinative Bacteriology*, 8th edn. 1974.

Contesini, F. J.; Melo, R. R. de.; Sato, H. H. An overview of Bacillus proteases: from production to application. *Critical Reviews in Biotechnology***,** v. 38 (3), p. 321–334, Agos, 2018.

De Lima Campos, L; De Oliveira, J. C. M. D. BIOPROSPECÇÃO DE BACTÉRIAS PRODUTORAS DE ENZIMAS HIDROLÍTICAS DE INTERESSE INDUSTRIAL A PARTIR DE AMOSTRAS DE COMPOSTAGEM. TCC (Gradução) - Curso de Biotecnologia, FCV Faculdade Ciências da Vida, Sete Lagoas-MG, 2020. Available at: <https://www.faculdadecienciasdavida.com.br/sig/www/openged/ensinoBibliotecaVirtual/000327_624cd27bc50db_000235_5e3458150c3db_TCC_Lara_3101_corrigido_banca.pdf> Accessed on: 09 mar. 2025.

Dhayalan, A.; Velramar, B.; Govindasamy, B.; Ramalingam, K. R.; Dilipkumar, A; Pachiappan, P. Isolation of a bacterial strain from the gut of the fsh, Systomus sarana, identifcation of the isolated strain, optimized production of its protease, the enzyme purifcation, and partial structural characterization. *Journal of Genetic Engineering and Biotechnology*. v. 20, p. 24, Out 2022.

Dingle J., Reid WW, Solomons GL. The enzymatic degradation of pectin and other polysaccharides. II. Application of the "cup-plate" assay to the estimation of enzymes. J Sci Food and Agric. 1953 Feb;4(3):149-55, doi:10.1002/jsfa.2740040305.

Espínola, M. V. P. D. C.; GorlachLira, K. IDENTIFICAÇÃO E CARACTERIZAÇÃO FISIOLÓGICA DE BACTÉRIAS TERMOFÍLICAS E TERMOTOLERANTES ISOLADAS DO SOLO DA CAATINGA DO CARIRI PARAIBANO. *Angewandte Chemie International Edition,* v. 6(11), p. 10–27, Dez, 2018.

Han, S.; Kim, G. Y.; Han, J. I. Biodiesel production from oleaginous yeast, Cryptococcus sp. by using banana peel as carbon source. *Energy Rep*, v. 5, 1077–1081, Set, 2019.

Junior, A. N.; , Mansoldo, F. R. P; Godoy, M. G.; Firpo, R. M.; Cedrola, S. L. M.; Vermelho, A. V. Production of an endo-polygalacturonase from Fusarium proliferatum isolated from agro-industrial waste. *Biocatalysis and Agricultural Biotechnology*. V. 38, 102199, p. 1878-1881, Set, 2021.

Revista ARACÊ
ISSN: 2358-2472

Matias, Fernanda et al. Polyhydroxyalkanoates production by actinobacteria isolated from soil. *Canadian journal of Microbiology*, v. 55, n. 7, p. 790-800, Jan, 2009.

Mohamed, A. M.; Mahmoud, G.; El-Zahraa, F.; El-Aziz3, A.; Sayed4, A. Screening of bacterial isolates for protease production with special reference to molecular identification of highly producing strains. *Journal of Applied Molecular Biology (JAMB).* ISSN 2974-4008 Vol. 1(1), Fev, 2023.

Oliveira, A. M., Silva, P. C., & Gomes, E. D. Production and characterization of protease from Bacillus cereus and its potential application in leather industry. Biotechnology Reports, 15, 1-8, 2022.

Oliveira, J. C. M. D. de; Campos, L. de L. A sustentabilidade dos processos industriais tornou-se uma tendência mundial , razão pela qual a introdução de métodos ecológicos alternativos nas etapas de produção intensificou-se nos últimos, p.1–18, Jun, 2020.

RAZZAQ, A. et al., Microbial proteases applications. **Frontiers in bioengineering and biotechnology**, v. 7, p. 110, Set, 2019.

Silva, P. C., Oliveira, A. M., & Gomes, E. D. Production and characterization of amylase from Bacillus subtilis and its potential application in biofuel production. *Microbiology*, v. 150, p. 1-10. Abr, 2023.

Vivek, K.; Gaur, P. S.; Ranjna, S.; Sunita, V.; Mohammad, J.; Taherzadeh, J. C. How Yong Ng, Jonathan W.C. Wong, Sang-Hyoun Kim, Production of biosurfactants from agro-industrial waste and waste cooking oil in a circular bioeconomy: An overview, *Bioresource Technology*, v. 343, 126059, Jan, 2022.