Methodological and Preparatory Implementation for the Analysis of the Microbiome of the Rhizosphere of Açaí *(Euterpe oleracea mart.)*

Joelma B. CARVALHO[1*], Elielson C. COSTA[1], Isabella C. SILVA[1], Maria S. R. BARBOSA[1], Letícia C. F. COSTA[1], Ângelo F. B. OLIVEIRA[1], Ian A. B. NUNES[1], Adan R. OLIVEIRA[1], Diego A. DAS GRAÇAS[1],

[1] *Biological Engineering Laboratory, Federal University of Pará/Institute of Biological Sciences, Belém, Brazil.*
** Corresponding author's email address: joelma.engbio20@gmail.com*

ABSTRACT

Açaí ***(Euterpe oleracea mart.)*** is a Brazilian fruit widely cultivated and consumed due to its antioxidant components and beneficial properties for human health. The spontaneous fermentation process of açaí is mediated by bacteria present on the surface of the fruit, constituting a characteristic microbiome. In this context, third-generation sequencers emerge as essential tools for investigating the genome, taxonomic and functional profile of these microbiomes. The methodology adopted consisted of collecting and scraping the outer layer of the fruit (mesocarp), followed by DNA extraction from the samples and integrity assessment. Subsequently, procedures were carried out to quantify the extracted material and verify its purity, using Qubit® and Nanodrop® equipment, following the protocols provided by the manufacturer and described in the scientific literature. In this way, the results obtained demonstrated satisfactory integrity, corroborating the results of the nanometric analysis of measurement and originality of the extracted content. The comparison with previous works reinforced the validity of the answers, guaranteeing the reliability of the results. Through this, the present research contributes to the advancement of scientific knowledge and advances in achieving the objective of characterizing the taxonomic and phylogenetic diversity of microorganisms in the three stages of açaí maturation, offering important insights for understanding the fermentation process and potentially beneficial to the food industry and human health.

Keywords: Açaí. PCR, Next-Generation Sequencing

1. INTRODUCTION

The ***Euterpe oleracea mart.*** fruit, popularly known as açaí and belonging to the Arecaceae family, has been cultivated for centuries in tropical Central and South America [1]. In Brazil, it has cultivars in some states such as Amazonas, Maranhão, Acre and, especially, in the state of Pará, where it is widely used in popular cuisine due to its unique flavor and health benefits, including its antioxidant components **[2].**

With the help of professionals from the Brazilian Agricultural Research Corporation (EMBRAPA, Belém), açaí bunches were selected and collected in the green, turning green and ripe stages, as established by previous references **[3].** Subsequently, the experimental and preparatory methods were applied for DNA integrity analysis and amplification, resulting in data corresponding to the literature base, being appropriate to proceed with sequencing.

In this way, we acquired knowledge about the microbial community and the taxonomic profile of each bacterial set present in the genetic material. Thus, the first and second stages of the process of characterizing the microbiological and phylogenetic diversity of the stages mentioned above showed development as predicted in the literature.

2. MATERIAL & METHODS

2.1 Collection of Açaí (Euterpe oleracea) Fruits and Scraping of the Pit

Following the references **[3]**, there are four stages of ripening of the *E. oleracea* fruit to be analyzed, however, only stages 2, 3 and 4 were considered due to the low accumulation of pulp in the first stage. With that, the selected stages were green, half-ripe and ripe. The pits were removed using latex gloves and placed in two Falcon tubes for each stage, later stored in closed styrofoam boxes and refrigerated at -21°C. Afterward, the mesocarp was removed with sterile scalpels under autoclaved aluminum foil, followed by weighing, with each sample containing 300 mg of material. The identification was carried out according to the ripening stage (F) and the corresponding replicate (R).

2.2 DNA Extraction and Electrophoresis

The DNA extraction process followed the guidelines of the FastDNA Spin Kit protocol provided by the manufacturer MD. During the electrophoresis step, 1% agarose gel was used, using 3 μL of sample for 30 mL of GelRed dye. The dilution of the density buffer was performed ten times. Then, the migration occurred with 10 nanograms of DNA at 100 W (Volts) with a duration of 5 minutes.

2.3 DNA Quantification Analysis and Sample Purity

At this point, the Thermo Scientific Qubit® equipment at 110 W is used, where the Eppendorf tubes containing the samples were incubated. The evaluation of the purity of the extracted content was carried out in the Thermo Scientific Nanodrop® apparatus. Pipette tips were used on a scale of 100 microliters. During the procedure, 1 mL of Elution Buffer (ET) and 1 mL of the sample were added. In this way, eighteen checks were performed.

3. RESULTS & DISCUSSION

3.1 DNA Extraction and Electrophoresis

Following the guidelines of **[5]** for the DNA extraction procedure, it is possible to verify that the experiment responses can supply the stipulated amount in each sample. Then, the integrity analysis of the contents obtained in the previous step was carried out. Among the eighteen samples, the seventh and fourteenth replicates stood out due to the greater accumulation of the genetic code. It was possible to verify this due to the color of the migration dye. On the other hand, the other samples presented small fractions during the migration of electrons on the plate.

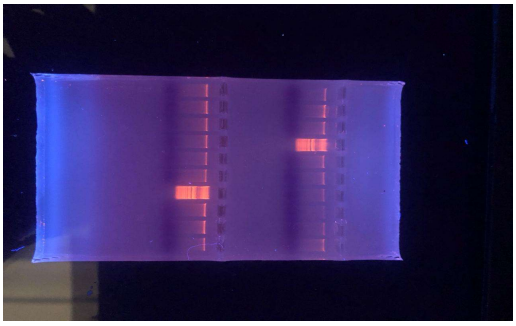

Figure 1 DNA integrity.

3.2 DNA Quantification and Sample Purity Analysis

In the DNA quantification, two pieces of equipment were used. Firstly, with the Qubit® quantifier using the absorbance in micrometers. Secondly, the Nanodrop® spectrophotometer was used, following the A260/A280 ratio used to evaluate the purity of DNA. In this way, it was possible to identify molecules and contaminants on the nanometric scale, obtaining more specific responses according to **[4]**.

Table 1 Qubit DNA Quantification Analysis

Samples	Quantification (ng/μL)
F2R1	6,4
F2R2	1,0
F2R3	1,2
F2R4	1,5
F2R5	1,0
F2R6	5,0
F3R1	4,3
F3R2	11,0
F3R3	2,4
F3R4	2,1
F3R5	5,9
F3R6	9,4
F4R1	4,7
F4R2	5,7
F4R3	7,7
F4R4	10,4
F4R5	9,4
F4R6	9,5

Source: Prepared by the author

According to the protocol guidelines, the Qubit® quantifier considers a concentration equivalent to 10 ng/μL to be appropriate, with much higher or lower values being considered contaminants in the samples. In this context, the results obtained in each experiment phase show quantities below those established by the protocol. Highlighting the replicates R1 and R6 with 6.4, 5.0 ng/μL, in phase 2, respectively, the replicates, R2, R5, and R6 with 11.0, 5.9, and 9.4 ng/μL in phase 3, and the

replicates R2, R3, R4, R5, R6 with 5.7, 7.7, 10.4, 9.4, and 9.5 in phase 4, respectively. It is important to emphasize that it is necessary to investigate on a smaller scale to include smaller substances and obtain more specific answers.

Table 2 Quantification Analysis and Purify by Nanodrop

Samples	Quantification (ng/μL)	Purity
F2R1	25,6	1,39
F2R2	19,6	1,46
F2R3	27,4	1,37
F2R4	20,2	1,36
F2R5	18,8	1,46
F2R6	17,3	1,49
F3R1	38,3	1,18
F3R2	58,7	1,15
F3R3	42,1	1,15
F3R4	37,0	1,15
F3R5	61,3	1,07
F3R6	66,2	1,10
F4R1	35,2	1,27
F4R2	33,7	1,25
F4R3	40,4	1,27
F4R4	43,2	1,25
F4R5	48,8	1,23
F4R6	46,9	1,25

Source: Prepared by the author

Compared to the previous results, we note a considerable and significant difference, since the spectrophotometer has greater precision to analyze the quantity and purity of the material. Therefore, the lowest concentration found was in replicate R6 of phase 2 with 17.3 ng/μL. The highest concentration was found in replicate R6 of phase 3, with 66.2 ng/μL The purity of the samples also varied, with the replicate of phase 2 with 1.10, the second lowest value presented in **(Table 2)**. In this bias, the prominent intermediate result was R2 of phase 3, with a concentration of 33.7 ng/μL, and a purity of 1.25. With this, the responses obtained are considered high when compared to the approximation of ~1.8 used for the classification of pure samples **[4].**

CONCLUSION

Through the methodological steps carried out on the ***E. oleracea*** fruit, sufficient results were obtained to proceed with the next experiments. The variation in the number of stages did not affect the efficiency of the experimental points. In addition, the verification with greater precision was fundamental to guarantee the purity of the contents, highlighting the quality of the samples to give continuity to the process and characterizing the microbiological diversity present in the three stages.

REFERENCES

[1] Laurindo, L. F., Barbalho, S. M., Araújo, A. C., Guiguer, E. L., Mondal, A., Bachtel, G., & Bishayee, A. (2023). Açaí (Euterpe oleracea Mart.) na saúde e na doença: uma revisão crítica. *Nutrientes*, *15*(4), 989.

[2] Silva, D. F. Custódio Neto da Silva, M. A. Rodrigues, G. M., Vidal, F. C. B. Barbosa, M. D. C. L. Oliveira Brito, L. M., ... & Soares Brandão Nascimento, M. D. D. (2020). Consumo de açaí (Euterpe oleracea Mart) e prevenção de doenças crônicas: existe associação? Um estudo preliminar. *Revista Scientific World*, *2020*.

[3] MOURA, Fábio Gomes et al. Biodiversidade bacteriana durante a fermentação espontânea de frutos de açaí (Euterpe oleracea). 2015..

[4] DA SILVA, Gabriela Fiori. Biomassa residual frutícola: uma abordagem metagenômica da microbiota autóctone e sua utilização na produção de biossurfactantes. 2023.

[5] SILVA, Rafael Correia da et al. Análise metagenômica da rizosfera de Campomanesia adamantium (Cambess) O. Berg. 2016.

ACKNOWLEDGEMENTS

Acknowledgments to the Biological Engineering Laboratory for providing the equipment used, to Prof. Dr. Diego Assis for his guidance and availability, and finally, to the laboratory partners