BAGHDAD JOURNAL OF BIOCHEMISTRY AND APPLIED BIOLOGICAL SCIENCES
2022, VOL. 3, NO. 02, 133-144, e-ISSN: 2706-9915, p-ISSN: 2706-9907
https://doi.org/10.47419/bjbabs.v3i02.123

ORIGINAL ARTICLE

Comparing protocols of DNA extraction from *Escherichia coli*: Analysis of purity and concentration by gel electrophoresis

Oslaene Alves de Brito[1], Francisca Alves dos Santos[1], Tassia Thais Al Yafawi[1], Cícero Roberto Nascimento Saraiva[1], Rakel Olinda Macedo da Silva[1], Lívia Maria Garcia Leandro[1], Pedro Everson Alexandre de Aquino[2], Dárcio Luiz de Sousa Júnior[3] and Maria Karollyna do Nascimento Silva Leandro[4]

[1]Doctor Leão Sampaio University Center, Juazeiro do Norte, CEP 63040-405, Brazil
[2]Federal University of Ceara, CEP 60020-181, Fortaleza, Brazil
[3]Federal University of Cariri, Crato, CEP 63130-025, Brazil
[4]Doctor Leão Sampaio University Center, Juazeiro do Norte, CEP 63040-405, Brazil

Received 15-04-2022
Revised 28-06-2022
Accepted 28-06-2022
Published 30-06-2022

Corresponding Author
Dárcio Luiz de Sousa Júnior
darciolsjr@gmail.com

Federal University of Cariri, Crato, CEP 63130-025, Brazil

DOI https://doi.org/10.47419/bjbabs.v3i02.123

Pages: 133-144

ABSTRACT

Background and objectives: Given the difficulty in establishing an ideal method that can successfully extract bacterial DNA of Gram-negative bacteria, the objective of this work was to compare the protocols of DNA extraction from *Escherichia coli* and to report the more practical, faster, and less costly ones.
Methods: *E. coli*, provided by Dr. Leão Sampaio University Center, was used. The methods used to extract bacterial DNA were: sodium dodecyl sulfate (SDS), salting out, cetyltrimethylammonium bromide (CTAB)/Phenol-Chloroform, and commercial kit for DNA isolation from Promega. The extracted DNA by all the methods was detected and assessed by the agarose gel electrophoresis.
Results: The best result was that obtained by the SDS method, it showed the greatest advantage for bringing speed, low cost, and good concentration of the extracted material. The other methods showed low-quality DNA, probably due to the presence of large amounts of proteins in the cell wall of *E. coli* interfering with the quality of the samples.
Conclusions: It was concluded that the SDS method can provide better DNA in terms of quality, which is preferred for the amplification by the polymerase (PCR) chain reaction. This will be helpful for diagnostic microbiologists because of the low cost and good quality of the extracted DNA.

Keywords DNA extraction, electrophoresis, Escherichia coli, SDS

INTRODUCTION

OPEN ACCESS

Based on Gram staining, the bacteria can be classified into Gram-positive and Gram-negative bacteria because of differences in the composition of their cell wall.[1] Gram-positive bacteria have a thick layer of peptidoglycan, making them more rigid; while Gram-negative bacteria have a thin layer of peptidoglycan, being more fragile and also an external membrane of lipopolysaccharide.[2]

Escherichia coli (*E. coli*) is a Gram-negative bacterium within the family Enterobacteriaceae. It can be described as a short, mobile or immobile bacillus that lives in aerobic and anaerobic environments. It naturally inhabits the intestine and is present in most urinary tract infections (UTIs).[3] The microorganisms' isolation methods have certain limitations regarding sensitivity and specificity, time of diagnosis, and treatment.[4] Earlier the diagnosis, the greater the probability of targeted treatment, the better the prognosis, and the lower the chance of selection of microorganisms resistant to broad-spectrum antibiotics.[5]

Prokaryotic cells have double-stranded and circular DNA, devoid of the nuclear membrane, proteins, and mitotic division. This genetic material has semiconservative replication, in addition, it has a plasmid as an extra chromosome, an optional structure related to the mechanism of resistance.[6] To obtain DNA, it must be intact, free of contaminants and interferents that may impair its analysis. The extraction methods consist of three steps: cell lysis and DNA solubilization, purification, and precipitation of this nucleic acid.[7]

The cell wall rupture is performed with detergents such as Sodium Dodecyl Sulfate (SDS) and Cetyltrimethylammonium Bromide (CTAB). DNA separation occurs in a heterogeneous environment with phenol, which denatures proteins and the genetic material is dissolved in only one phase.[8] To remove the proteins, high salt concentrations are added (salting-out), so that they are separated from the DNA, which will be precipitated with alcohol (ethanol or isopropanol) to guarantee its purity. The analysis of DNA purity in sufficient quality and quantity can be verified by methods such as fluorimetry, spectrometry, and electrophoresis.[9] Electrophoresis is a simple technique that uses electric current to promote the separation of charged molecules, such as proteins and nucleic acids. This technique has been increasingly used in the laboratory routine contributing to the diagnosis and prognosis of several diseases.[10]

Given the difficulty of microbial diagnostics, through traditional cultivation methods; the extraction of genetic material with low cost, fast and effective techniques became important to optimize techniques in the area of molecular genetics. Thus, this study aims to compare bacterial DNA extraction protocols to select a method that offers the best cost benefit and better DNA quality.

MATERIALS AND METHODS

Sample preparation

Bacteria from the standard strain of *E. coli*, grown in Brain Heart Infusion (BHI), was transferred after a 24-hour incubation period to a 2 mL Eppendorf microtube. After transfer, the samples were centrifuged at 10000 rpm for two minutes and stored at a temperature

of -20ºC for further DNA extraction.

Extraction methods

In this work, methods used are Sodium Dodecyl Sulfate (SDS), CTAB/Phenol-Chloroform, Salting-out [with and without Proteinase K enzyme (different time points and different temperatures were used to evaluate the activity of the Proteinase K)], and Wizard® Genomic DNA Purification Kit from Promega (for Isolating Genomic DNA from Gram Positive and Gram Negative Bacteria).[4]

Extraction by SDS

In tubes containing the previously obtained bacterial pellet, 500 μL of lysis buffer (400mM Tris-HCl and EDTA, 50 mM, 500mM NaCl, 1% SDS) was added. The tubes were vortexed vigorously for 15 seconds and then centrifuged for 5 minutes at 10,000 rpm. After that, the supernatant was transferred to a new labeled tube and 1 mL of ice-cold concentrated ethanol was added. The tubes were gently shaken by inversion and centrifuged for 5 minutes at 10,000 rpm. The supernatant and the pellet were washed with 700 μL of ice-cold 70% ethanol followed by centrifugation for 5 minutes at 10,000 rpm. After discarding the supernatant, the tubes were placed inverted on absorbent paper to dry the pellet for approximately 30 minutes. DNA was resuspended in 50μL of ultrapure H_2O and the sample was stored at -20°C until further use.[11]

Extraction with CTAB/Phenol-Chloroform

This method was performed according to Oliveira (2006),[12] where sterile 1.5 mL micro-tubes containing the bacterial pellet, as previously described, were added 250 μL of TE (10mM Tris HCl pH 7.6; 1mM EDTA) and subsequent addition of 100 μL of 5M sodium chloride (NaCl) and 100 μL of cetyltrimethylammonium bromide (CTAB) preheated to 65°C. The suspension was stirred for ten seconds and incubated in water bath at 65°C for ten minutes. Then, 750 μL of phenol/chloroform (1:1) were added and the mixture was shaken gently by inversion for ten seconds and then by centrifugation as described by Oliveira (2006).[12] Another wash was made with 200 μL of chloroform-isoamyl alcohol (24:1) and centrifugation. The DNA was precipitated with 1/10 volume of 3M sodium acetate and 3 times the volume of concentrated ethanol. After that, it was homogenized by inversion and incubated overnight at -20°C. These were then centrifuged for twenty minutes at 15,000 rpm, the supernatant was discarded, and 500 μL of 70% ethanol at room temperature was added. Washing with 70% ethanol was repeated and the pellet dried at 56°C in an oven. After ethanol was completely evaporated and the DNA solubilized in 50 μL of ultrapure water. The tubes were left for 20 minutes at room temperature and then stored at -20°C.[13]

Extraction by salting-out

Salting-out without Proteinase K (for 1h at 60ºC)

Two hundred microliters of TE (10mM Tris HCl pH 7.6; 1mM EDTA) plus 0.6% SDS was added to the 1.5 mL polypropylene tubes containing the *E. coli* and then incubated in a water bath for 1 hour at 60°C. After incubation, 35 μL of NaCl (5M) was added, shaken manually and then centrifuged for 2 minutes at 13,000 rpm. The supernatant was transferred to a new tube and 400 μL of ice-cold absolute ethanol was added. The tubes were shaken by inversion and centrifuged for 10 minutes at 13,000 rpm. The supernatant was discarded and 1 mL of 70% ethanol was added, and the mixture was again centrifuged for 10 minutes at 13,000 rpm, discarded the supernatant, and repeated washing with 70% ethanol once more. The supernatant was discarded and the tubes were placed inverted on absorbent paper for 30 minutes until the residual ethanol evaporated. The pellet was resuspended in 50 μL of autoclaved ultrapure water and then stored at -20°C until further use.[14]

Salting-out with Proteinase K (for 1h at 60ºC)

The method was performed with the same protocol of salting-out without Proteinase K, but with the addition of 10μL of Proteinase K with TE (Tris HCl 10mM pH 7.6; EDTA 1mM) plus SDS 0.6%.

Salting-out with Proteinase K (for 2h at 42°C)

The method was according to Abrão et al (2005)[14] with some modifications as follows. In most of the DNA extraction protocols that use the enzyme Proteinase K (PK), the incubation time is 2 hours at 42°C, which makes the method too long. So to optimize the run time of this methodology, we used the incubation time at 60°C for 1 hour and the 2h time at 42°C and thus evaluate the effectiveness of the enzyme at a higher temperature and shorter time. 200μL of TE (10mM Tris HCl pH 7.6; 1mM EDTA) plus 0.6% SDS was added to 1.5 mL microtubes containing the Staphylococcus aureus bacteria and 5 μL of PK (10mg/mL). The samples were incubated in a water bath for 1 h at 60°C and 2 h at 42°C at separate times. After incubation, 35μL of 5M NaCl was added and subsequently, the tubes were shaken vigorously. The subsequent steps were performed as described above.

Extraction with Promega kit

One mL of the bacterial suspension, from the culture obtained as described above, was centrifuged for 2 minutes at 13,000 rpm. The supernatant was discarded after centrifugation. The pellet obtained was resuspended in 480 μL of TE buffer (10mM Tris HCl pH 7.6; 1mM EDTA) and then 600 μL of Nuclei Lysis Solution and 10 μL of PK were added and resuspended gently with a micropipette. Subsequent steps were performed according to the

manufacturer's instructions.[15]

Evaluation of DNA yield and its integrity

The integrity of DNA obtained was analyzed by electrophoresis in 1% agarose gel, where 8 μL of the sample was mixed in 0.5 μL of intercalante Blue Green Loading Dye I (LGC Biotechnology) and after running at 70 volts per 30 minutes and visualized in transilluminator. To evaluate the yield of the purified DNAs, the DNA Ladder 1 Kb marker (KASVI) was used.

RESULTS

The comparison between the protocols tested was performed by the amount of final DNA extracted from the *E. coli* samples in each protocol tested. The results can be observed in Figure 1, which presents the quantities and quality of the extracted DNA samples. The protocol that obtained the highest concentration of DNA (ng/μL) was the method SDS, which is the one of highlights of being a fast and simple method. The protocol that provided the extraction of DNA in lesser concentration was the Kit Promega (6).

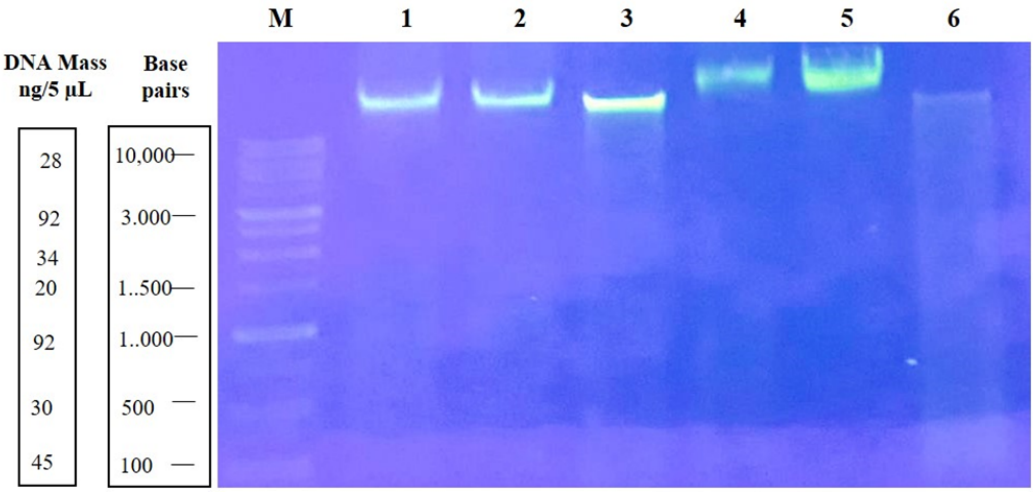


Figure 1 Evaluation of six DNA extraction methods of *Escherichia coli*. Channels: (M) Ladder DNA marker 1 kb; (1) Salting-out method incubated for 1 hour at 60°C without proteinase K; (2) Salting-out method incubated for 1 hour at 60°C with Proteinase K; (3) Sodium dodecyl sulfate (SDS) method; (4) Salting out method incubated for 2 hours at 42°C with Proteinase K; (5) CTAB/Phenol/CAI method and (6) Promega kit.

Table 1 shows the results obtained after agarose gel electrophoresis 1% for quantification of the extracted DNA. For the evaluation of the DNA yield, it was compared to the KASVI DNA Ladder 1KB marker, which contains its mass in 5 ng/μL known, thus having as a base to calculate the samples according to the quantity inserted in the gel.

Table 1 **Concentrations obtained through comparative analysis with DNA ladder.**

Protocol	DNA concentration	
	ng/7 μL	ng/μL
SDS	322	46
CTAB/Phenol-Chloroform	257.6	36.8
Salting-out without PK (for 1h at 60ºC)	257.6	36.8
Salting-out with PK (for 1h at 60ºC)	257.6	36.8
Salting-out with PK (for 2h at 42ºC)	257.6	36.8
Promega kit	25.2	3.6

PK, *proteinase K;* ***SDS,*** *sodium dodecyl sulfate*

DISCUSSION

According to Amaral et al. (2016),[16] the SDS method is more efficient in terms of time and quality of the extracted DNA, besides presenting satisfactory concentration and low cost, being accessible for the development of research in several laboratories. In the study of Baratto and Megiolara (2012),[17] when comparing the DNA extraction by the SDS method of Gram-positive and Gram-negative bacteria, different results were obtained regarding the quality of the samples, suggesting that the distinct composition of the cell wall of these microorganisms may directly interfere with the test since there is a greater difficulty with Gram-positive bacteria, thus requiring more complex methodologies to obtain their genetic material.

In the present study, the protocol that provided the DNA extraction in lower concentration was using the Promega kit, not corroborating with Lipay and Bianco (2015),[18] since they report that the commercial kits for extraction of DNA present with greater frequency, better quality and purity than the manual methods, being the only disadvantage corresponding to the values of the tests.

Salting out without Proteinase K for 1 hour at 60°C and with Proteinase k 1 hour at 60°C and 2 hours at 42°C have obtained similar results, although with the use of Proteinase K the test becomes more expensive, and the temperature decrease and time increase makes the test more time-consuming, being more effective, practical and fast the method Salting out without Proteinase K 1 hour at 60°C.

The protocols with Proteinase K and CTBA are widely used and give good results. However, the use of phenol and chloroform are toxic to the environment and dangerous when handled, thus indicating their replacement by saline solution.[19] According to Bonatto and Breve (2014),[2] SDS extraction of *E. coli* DNA is effective in obtaining genomic DNA, but for plasmodial DNA extraction, the results are not so favorable.

The SDS is a detergent agent that solubilizes lipids, is a denaturant of proteins and also breaks non-covalent inter-subunit bonds of oligomeric proteins. It is also used to make sure that no nuclease that does not depend on Mg^{+2} causes any degradation. Commercially available kits, in general, result in good quality purifications for use in the most varied molecular techniques, including PCR. However, its main disadvantage is the high cost per

sample.[20]

The CTBA/phenol-Chloroforic test resulted in the same concentration obtained with salting out, however, is a test that presents a higher cost due to the need to use two reagents: phenol and chloroform. In addition, it has as disadvantage the risk to those who administer these substances, it is toxic to health.[19]

Andreatti Filho et al. (2011)[21] verified after extracting *Salmonella* DNA, that the material obtained by CTAB presented a higher quality of sharpness (without the formation of non-specific bands) than that obtained by the SDS method, and quality similar to that of the product extracted by the phenol-chloroform method, but with less intensity compared to the extraction method with guanidine thiocyanate.

Phenol causes protein denaturation efficiently. Chloroform is also used as a denaturing agent for the proteins contained in the sample. The mixture of phenol and chloroform is very efficient to deproteinize and its action is based on the hydrophobic property of proteins that have an affinity for organic solventes.[13]

CONCLUSIONS

The extraction by the SDS method obtained DNA of better quality, satisfactory for polymerase chain reaction (PCR). It is a time-efficient method, that presents well quality of the extracted DNA, satisfactory concentrations, and low cost being accessible for the development of research in several laboratories. The other tested methods, presented low-quality DNA, although with good performance except with the Kit Promega, which can be explained by the presence of a large number of proteins in the *E. coli* cell wall, thus presenting the presence of contaminants by proteins in the electrophoresis analysis, interfering in the quality of the samples. Despite the results obtained, using different bacterial DNA extraction methodologies, it is suggested that further studies are needed. Despite the great advances in the area, still has a wide field to be explored that can generate many benefits in microbial diagnosis, with low cost and good effectiveness.

ABBREVIATIONS

BHI, brain heart infusion; CTAB, Cetyltrimethylammonium Bromide; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; HCl, hydrochloric acid; SDS, sodium dodecyl sulfate; PCR, polymerase chain reaction; Tris, tris(hidroximetil)aminometano.

ACKNOWLEDGMENTS

Nil.

DECLARATIONS

Authors' contributions

Conceptualization: OAB, MKNSL, FAS. Data curation, formal analysis, investigation, methodology, project administration, resources, and writing-original draft: OAB, MKNSL, LMGL. Funding acquisition: MKSNL, DLSJ, LMGL. Software: OAB, TTAY, LMGL. Supervision: MKNSL, LMGL, ROMS. Validation and visualization: PEAA, DLSJ, ROMS. Writing-review & editing: DLSJ, CRNS, FAS.

Conflict of interest

The authors declare no conflict of interest.

Ethical approvals

Not applicable.

Data availability

Date is available from corresponding author upon reasonable request.

Funding resources

No external fund was received.

REFERENCES

1. Miranda RR, Monteiro FC, Boreiko S, Bittencourt JVM. Detergents and ultrasound evaluation for genomic DNA isolation of food bacteria. Rev Bras Tecnol Agroind. 2011;5(01):398–407. Available from: 10.3895/S1981-36862011000100003S1.
2. Bonatto FDA, Breve HAM, Tejada ECS. Comparison of DNA extraction protocols in bacteria E. coli and Pseudomonas spp. Rev Electron Anal Clin. 2014;2(01):1–11.
3. Eusébio A, Araújo C, Andrade M, Duarte A. Escherichia coli in community urinary tract infections: commensal or pathogenic? Acta Urol Port. 2016;33(02):37–42. Available from: 10.1016/j.acup.2016.02.002.
4. de Sousa Alves PG, Silva JC, Lopes TP, Tavares DSA, Linhares ML, de Sousa Júnior DL, et al. Optimization of Gram-positive bacteria DNA extraction protocols and purity and concentration analysis by gel electrophoresis. Rev Interfaces (Juazeiro do Norte). 2021;9(02):1133–1140. Available from: 10.16891/944.
5. Nguyen HB, Rivers EP, Abrahamian FM, et al. Severe sepsis and septic shock: review of the literature and emergency department management guidelines. Ann Emerg Med. 2006;48(1):28–54. Available from: 10.1016/j.annemergmed.2006.02.015.
6. Reinhardt G, Walflor HSM, Trigo JHB, Ribas JLC. Development and applications gene vaccines for treatment and diseases prevention. Rev Saude Desenvolv. 2017;11(7):245–262.

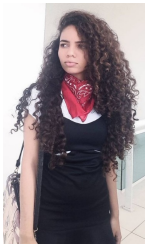
7. Caldart ET, Chiappetta CM, Lopes EF, Ravazzolo AP. Comparative analysis of genomic DNA extraction methods from small ruminant blood and milk cells. Acta Sci Vet. 2011;39(01):945.
8. Saba F, Papizadeh M, Khansha J, Sedghi M, Rasooli M, Amoozegar MA, et al. A rapid and reproducible genomic DNA extraction protocol for sequence-based identification of archaea, bacteria, cyanobacteria, diatoms, fungi, and green algae. J Med Bacteriol. 2017;5(3-4):22–28.
9. Yuan S, Cohen DB, Ravel J, Abdo Z, Forney LJ. Evaluation of methods for the extraction and purification of DNA from the human microbiome. PLoS One. 2012;7(3):33865–33865. Available from: 10.1371/journal.pone.0033865.
10. de Oliveira E, de Campos Trentin T, Camargo F, Pinto YDP, Martins DB. Electrophoresis: concepts and applications. Enciclopédia Biosfera. 2015;11(22):1129–1149. Available from: 10.18677/Enciclopedia_Biosfera_2015_149.
11. Júnior WPL, Sampaio R, da Silva GR, Granja F, de Lima Júnior MM. Comparison between three methods of DNA extraction from urine. In: XI Semana Nacional de Ciência e Tecnologia em Roraima: SNCT-RR; 2017. .
12. de Oliveira DE, Bacchi MM, Macarenco RSS, Tagliarini JV, Cordeiro RC, Bacchi CE. Human papillomavirus and Epstein-Barr virus infection, p53 expression, and cellular proliferation in laryngeal carcinoma. Am J Clin Pathol. 2006;126(2):284–293. Available from: 10.1309/UU2JADUEHDWATVM9.
13. de S Oliveira MC, de A Regitano LC, Roese AD, Anthonisen DG, Patrocinio ED, Parma MM, et al. Fundamentos teóricos-práticos e protocolos de extração e de amplificação de DNA por meio da técnica de reação em cadeia de polimerase. Embrapa Southeastern Livestock; 2007.
14. Abrão MG, Billerbeck AEC, Nishi MY, Marui S, de Mendonça BB. Standardization of DNA extraction with NaCl from oral mucosa cells: application in PROP1 gene study. Arq Bras Endocrinol Metab. 2005;49(6). Available from: 10.1590/S0004-27302005000600019.
15. Promega. Wizard® Genomic DNA PurificationKit: Technical Manual;. Available from: shorturl.at/ersU3.
16. do Amaral CB, Vieira NA, Ândrea Renata da Silva R, dos Santos FJ, Campos BA, de Oliveira SL, et al. Evaluation protocols for the extraction of genomic DNA from bovine blood. J Selva Andina Res Soc. 2016;7(02):95–103.
17. Baratto CM, Megiolaro F. Comparison of different bacterial DNA extraction protocols by using RAPD-PCR. Unoesc & Ciência - ACET. 2012;3(01):121–130.
18. Lipay MVN, Bianco B. Biologia molecular: Metodos e Interpretacao. 1st ed. Rio de Janeiro: Roca; 2015.
19. de Cássia Ribeiro Vieira B, Alfaiate MB, Oliveira APG, de Fátima Madella-Oliveira A, Quirino CR, Souza MH, et al. Metodologies used in the extraction of deoxyribonucleic acid. Pubvet. 2015;9(01):25–37.
20. Araujo FR, Ramos CADN, Luíz HL, Péres IAHFS, Oliveira RHMD, Souza IIFD, et al. Evaluation of a genomic DNA extraction protocol from whole blood. In: Comuni-

cado Técnico. Embrapa Gado de Corte, 120; 2009. .
21. Filho RLA, Gonçalves GAM, Okamoto AS, de Lima ET. Comparison of DNA extraction methods in polymerase chain reaction for the detection of Salmonella enterica serovar enteritidis in poultry products. Ci Anim Bras. 2011;12(1):115–119. Available from: 10.5216/cab.v12i1.3774.

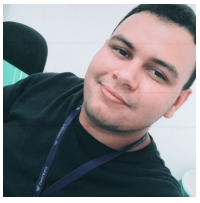
AUTHOR BIOGRAPHY

Oslaene Alves de Brito is a Biomedical Scientist and Technical Manager at the Municipal Laboratory of Tupanatinga, attends the LAMAB Clinic as a Clinical Cytologist, and teaches at the Centro de Ensino Superior do Vale do São Francisco (CESVASF).

Francisca Alves dos Santos is a Molecular Biology Laboratory Technician at UNILEÃO University Center and co-supervisor of BioGem-Liga Academic League of Genetics and Molecular Biology (UNILEÃO).

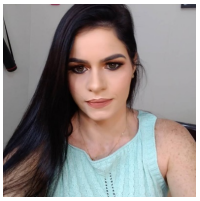


Tassia Thais Al Yafawi holds a B.Sc. in Biomedicine and M.Sc. in Biological Chemistry. Currently, she is a Coordinator of the Microbiology and Molecular Biology, Urinalysis and Parasitology sectors at the Laboratório de Análises Clínicas Vicente Lemos.



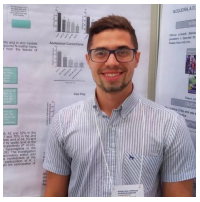
Cícero Roberto Nascimento Saraiva has a bachelor of Biomedicine from the Leão Sampaio University Center, Juazeiro do Norte, CEP (2015). Specialist in Clinical Hematology, by the Leão Sampaio University Center - Juazeiro do Norte - CE (2017). Master in Biological Chemistry, at the Regional University of Cariri, Crato (URCA) (2019). He served as preceptor of Supervised Internship I in the Hematology (2019) and Urinalysis and Parasitology (2019) sectors, and in the Supervised Internship II in the Immunology and Coagulogram sector (2016-2018) in the Biomedicine course at the Leão Sampaio University Center and was an Analyst and Responsible for the Immunology and Coagulation Sector of the Laboratory School of Clinical Analysis of the Leão Sampaio University Center (2016-2018). He is currently a Full Professor of the Disciplines of Applied Chemistry I and Applied Chemistry II and Advisor of the Academic League of Immunology (LAI), at the

Leão Sampaio University Center. Co-supervisor of the Scientific Initiation Program. His experience includes the research on the chemistry of natural products and the teaching of chemistry in undergraduate courses in the health area.



Rakel Olinda Macedo da Silva has a B.Sc. in Biomedicine from Leão Sampaio University Center (2012) and a specialization in Clinical Hematology from the same institution in 2015. Her specialization in Higher Education Teaching from Integrated Faculties of Patos was approved in 2016. Her master's degree in Molecular Bioprospecting, with emphasis on Microbiology at the Regional University of Cariri. She has experience in the areas of Clinical Analysis, focusing on Clinical Microbiology, and currently works as a Professor of Biosafety (since 2016) and Microbiological Analysis of Water (since 2015) and Preceptor of the Microbiology sector in the Supervised Internship I at Leão Sampaio University Center (since 2013). Advisor of LAMIC- Academic League of Clinical Microbiology of UNILEÃO (since 2016).

Lívia Maria Garcia Leandro received her B.Sc. in Biomedicine from Centro Universitário Leão Sampaio in 2013. She works as a clinical analyst at Laboratório Vicente Lemos. She specializist in Clinical Hematology and Clinical Microbiology. She has experience in the fields of Quality Control, Clinical Hematology, Urinalysis, Clinical Parasitology, Clinical Biochemistry, and Microbiology. Co-advisor at the Academic League of Clinical Microbiology (LAMIC) at the Leão Sampaio University Center.



Pedro Everson Alexandre de Aquino graduated in Biomedicine from the Leão Sampaio University Center-Juazeiro do Norte-CE and specialist in Clinical Hematology from the same institution. He holds a master's and a doctorate in Pharmacology from the Federal University of Ceará and is a member of the Neuropharmacology Laboratory at the Drug Research and Development Center (NPDM). He is one of the Associate Members of the Brazilian Society of Physiology (SBFIs) and the Brazilian Society of Neuroscience and Behavior (SBNeC). He develops research projects in the areas of Pharmacology with an emphasis on Neuropharmacology, Inflammation, Microbiology, and Natural Products.

Dárcio Luiz de Sousa Júnior holds a B.Sc. in Biomedicine from Centro Universitário-UNILEÃO (2014) and M.Sc. in Biological Chemistry at the Regional University of Cariri, with a Specialization in Clinical Microbiology at Centro Universitário - UNILEÃO (2016) and Specialization in Clinical Pharmacology at the Regional University of Cariri (2018). He has research experience in the field of Pharmacology and Microbiology of Natural Products, with an emphasis on modulating antimicrobial activity and animal models of inflammation and nociception. He currently works with alternative models (Drosophila melanogaster and Zebrafish) with an emphasis on evaluating the toxicity of natural products.

Maria Karollyna do Nascimento Silva Leandro Graduated with a B.Sc. in Biomedicine (2015) from the Doctor Leão Sampaio University Center, UNILEÃO. Specialization in Clinical Microbiology (2016) from the Leão Sampaio University Center, UNILEÃO. She has a M.Sc. in Molecular Bioprospection (Research line: Microbiology and Natural Products) from the Regional University of Cariri, URCA (2018). Her research is mainly on the antimicrobial activity of natural products and nanostructured materials associated with phototherapy. Preceptor of the Biochemistry and Parasitology sectors and professor of the disciplines of Clinical Mycology, and Molecular Genetics at UNILEÃO.