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Acute Toxicity Assessment of Rhamnolipid Biosurfactant Produced by *Pseudomonas aeruginosa* BM02 Using Andiroba (*Carapa guianensis* Aubl.) Waste in *Pithecopus hypochondrialis* (Anura: Hylidae) Tadpoles

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Abstract

Rhamnolipid biosurfactants are amphiphilic molecules synthesized predominantly by the bacterium *Pseudomonas aeruginosa*, which can utilize a variety of substrates for growth, including agro-industrial waste. This study aimed to evaluate the toxicity of biosurfactants synthesized by *P. aeruginosa* BM02, utilizing andiroba (*Carapa guianensis* Aubl.) (Sapindales: Meliaceae) waste as the sole nutrient source, on *Pithecopus hypochondrialis* (Daudin, 1800) (Anura: Hylidae) tadpoles. The biosurfactant was produced in a vegetable saline medium, achieving maximum emulsifying activity of 72% and a yield of 2.5 g/L^{-1} on the eleventh day of fermentation. Chemical characterization confirmed its identity as rhamnolipid. Tadpoles exposed to the biosurfactant (0.5 mg L^{-1}) showed a slight reduction in key morphological parameters compared to the control group. The control cohort measured 17.87 mm in total length, with head and tail lengths of 3.17 mm and 12.84 mm, respectively, while tadpoles exposed to the biosurfactant measured 15.30 mm, 2.81 mm, and 10.62 mm. These reductions could reflect adaptive responses to environmental stressors or intrinsic alterations in growth rates and metabolism. Notably, no significant behavioral alterations were observed, including impairments in foraging and swimming behavior, suggesting that biosurfactant exposure does not adversely affect the overall well-being of the tadpoles. Histological analysis revealed healthy epithelial and muscular tissues, indicating that structural integrity was maintained post-exposure. The absence of hyperplasia in intestinal columnar cells suggests preserved digestive functionality, and no acute inflammatory response was detected in the gut environment. Collectively, these results indicate that exposure to 0.5 mg L^{-1} of the biosurfactant does not induce significant adverse effects on tadpole survival, highlighting a favorable safety profile for this biosurfactant in the context of the study.

Keywords: Amazonian fruits. Amphibians. Ecotoxicology. Histopathology. Microbial surfactants.

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INTRODUCTION

Biosurfactants have emerged as significant amphiphilic molecules with various biological and biotechnological applications, particularly due to their ability to reduce surface and interfacial tension. (Sá *et al*., 2025). These compounds are synthesized by microorganisms that utilize diverse nutrient sources for growth. (Kitamoto *et al*., 2009). Among the different biosurfactant types, rhamnolipids are the most extensively researched subclass, primarily produced by *Pseudomonas aeruginosa*, a versatile Gram-negative bacterium known for its metabolic versatility and ability to utilize diverse substrates (Santos *et al.*, 2024).

The bioavailability of nutrients in the culture medium significantly influences bacterial growth and subsequent biosurfactant yield (Zhang *et al*., 2021). *P. aeruginosa* can metabolize different organic substrates for biosurfactant biosynthesis, including soybean oil, corn oil, and agro-industrial waste (Pérez-Armendáriz *et al.*, 2019). Notably, investigations by the Group of Studies in Microbial Technologies (GETAM) at the Federal University of South and Southeast Pará (Unifesspa) in Marabá-PA, Brazil, have identified several Amazonian plant waste materials – such as açai, andiroba, babassu, Brazil nuts, and cacao – as promising substrates for biosurfactant production. Among these, andiroba (*Carapa guianensis* Aubl.) (Sapindales: Meliaceae) has emerged as a particularly effective nutrient source owing to its distinct chemical profile (Sá *et al*., 2025).

Recent research has underscored the potential applications of bacterial biosurfactants across multiple industrial sectors. Zhu *et al*. (2024) and Yang *et al*. (2024) have documented the efficacy of biosurfactants in marine conservation, particularly for oil-contaminated site bioremediation and plastic degradation in coastal sediment. In the medical field, biosurfactants are being explored for applications in drug delivery systems, tumor cell differentiation and apoptosis induction, treatment of bacterial and viral infections, wound healing, and immunomodulation. (Wang *et al*., 2024). Furthermore, findings by Zamorano-González *et al*. (2025) and Sá *et al*. (2025) propose the potential for biosurfactants to serve as biocontrol agents in managing pest populations and arbovirus vectors.

Despite their environmentally friendly characteristics, marked by low or negligible toxicity towards humans and animals, and high biodegradability, comprehensive studies validating the toxicological safety of biosurfactants remain paramount. The U.S. Food and Drug Administration underscores the need for a thorough investigation into the potentially toxic effects of natural products with biological, therapeutic, and biotechnological potential, such as biosurfactants (Zygmuntowicz *et al*., 2020).

Environmental toxicity from organic compounds poses significant threats to amphibian populations (Slaby *et al.*, 2019). Amphibians, as vital components of ecological stability, are a transitional group between aquatic and terrestrial ecosystems, making them ideal laboratory models for testing potential contaminants (Langlois, 2021). The species *Pithecopus hypochondrialis* (Daudin, 1800) (Anura: Hylidae) is a widely distributed leaf frog inhabiting diverse ecological habitats in Brazil, Colombia, French Guiana, Guyana, Suriname, and Venezuela, predominantly found in forest clearings, such as the Amazon rainforest (Magalhães *et al*., 2024). Its ecological characteristics make it an effective bioindicator for assessing the toxicity of organic compounds.

To contribute to the scarce literature available, the present study aimed to evaluate the toxicity of biosurfactants produced by P. aeruginosa BM02, utilizing andiroba waste as the sole nutrient source, against the anuran amphibian *P. hypochondrialis*.

MATERIAL AND METHODS

Ethical considerations

This study did not require ethical approval for human subjects, as it exclusively involved bacterial strains and did not utilize any human participants or cell lines. The research focused on the production of biosurfactants using these bacterial strains. However, the use of the anuran amphibian *P. hypochondrialis* was sanctioned by the Ethics Committee on the Use of Animals at the Unifesspa under registration number CAAE 23479.006811/2024-89.

Biological materials

Bacterial strain

The rhamnolipid-producing bacterium, *Pseudomonas aeruginosa* BM02 (GenBank OP410927.1), was isolated from surface soil at a mining site in Pará (3°14'47.604"S 47°44'12.981"W), as reported by Santos *et al.* (2024). This strain is registered in the Brazilian System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number A4DA401 and is preserved in a glycerol solution at -20 °C within the Bioassay and Bioprocess Laboratory (L@βio) at Unifesspa in Marabá-PA, Brazil.

Amazonian fruit waste

Andiroba (*Carapa guianensis* Aubl.) waste, a byproduct of artisanal oil extraction, was provided in its raw state by the Praialta and Piranheira Agroextractive Settlement Project, located in Nova Ipixuna, PA,

Brazil. This waste was utilized as the sole nutrient source for rhamnolipid biosurfactant production, as outlined by Sá *et al*. (2025), and is documented under SisGen registration number A27D6F4.

Pithecopus hypochondrialis

Specimens of *Pithecopus hypochondrialis* (Daudin, 1800) (Anura: Hylidae) were collected using traps placed within vegetation near temporary pools along the Tauarizinho River (5°21'42.4"S 49°01'27.8"W), situated in Unit III of Unifesspa, Marabá-PA, Brazil. The characteristics and constituent compounds of the sampled water are summarized in Table 1. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were measured following the methodologies outlined by the American Public Health Association (APHA, 2005). The concentration of NH_2/NH_{4+} was assessed using the indophenol blue colorimetric method described by Scheiner (1976). pH levels were recorded utilizing a multiparameter probe (HANNA model HI9811-5, Barueri, Brazil), while turbidity was determined in a turbidimeter (model TB-1000, Joinville, Brazil). This collection method was employed to promote spawning in controlled plastic containers (Figure 1). Following incubation, the tadpoles were reared separately in aquariums with dechlorinated water (Alcon®, São Paulo, Brazil) maintained at room temperature (25 ± 2 °C).

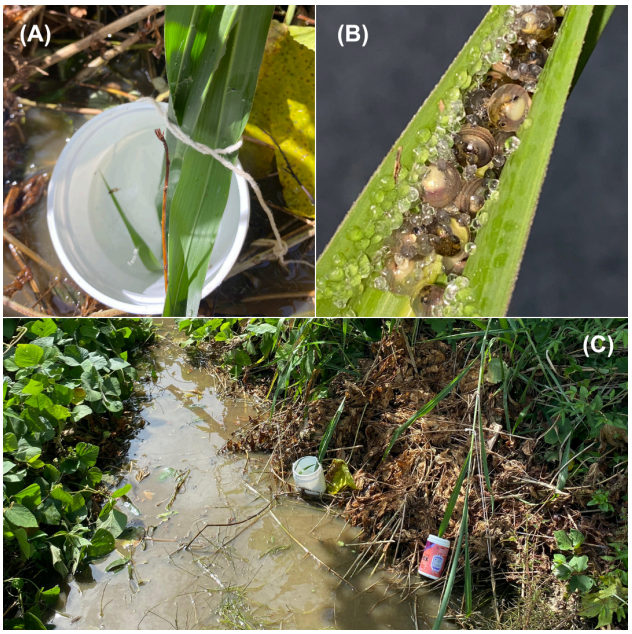

Figure 1. Specimen collection of *Pithecopus hypochondrialis*. **(A)** Egg collection trap, **(B)** foliage containing *P. hypochondrialis* embryos, and **(C)** traps positioned adjacent to temporary aquatic habitats along the Tauarizinho River.

Production and extraction of biosurfactant

The biosurfactant was produced in Erlenmeyer flasks containing a vegetable saline medium (VSM) composed of (0.05 g L^{-1}) K_2HPO_4; (0.05 g L^{-1}) KH_2PO_4, and andiroba waste as the sole nutrient source, optimized for microbial growth (adapted by Sá *et al*., 2025). The formulation is protected under industrial confidentiality, with an Invention Patent (BR1020230146740) filed with the Brazilian Institute of Industrial Property. The medium's pH was adjusted to 7.0, and then sterilized at 121 °C for 30 minutes. After cooling, a 5% of a bacterial inoculum, exhibiting an optical density at 600 nm ranging from 0.6 to 0.8 – measured using a Bel V-M5 Visible Spectrophotometer (Biovera, Germany) –, previously cultivated in Luria Bertani broth (LB; Kasvi, Pinhais, Brazil), was transferred into the flasks. The flasks were incubated in an orbital shaker (SL-22, Solab, São Paulo, Brazil) at 30 °C and 180 rpm for 19 days.

Post-incubation, the bacterial culture was centrifuged (SL-700, Solab) at 4500 rpm for 15 minutes to obtain the cell-free broth (CFB). The CFB was then sterilized and subjected to extraction using a chloroform-methanol solution (3:1, v/v) in a separatory funnel. The mixture was stirred and allowed to settle for phase separation to occur. The hydroalcoholic phase was discarded, and the organic phase was collected. This extraction process was performed thrice to increase yield. Finally, the organic phase was evaporated using a rotary evaporator (LGI-52CS-1, Scientific, São Paulo, Brazil) and dried in an air-circulated oven until a consistent weight was achieved.

Quantification and characterization of biosurfactant

The rhamnolipid biosurfactant concentration was quantified utilizing the orcinol method, which effectively measures rhamnose levels (Camilios Neto *et al*., 2008). Specifically, 60 µL of a 10 mg mL^{-1} biosurfactant suspension was combined with 14.96 mL of a solution containing 0.19% orcinol in 53% H_2SO_4. The mixture was incubated at 80 °C for 30 minutes and subsequently allowed to equilibrate at ambient temperature (25 ± 2 °C) for 15 minutes. Absorbance was measured spectrophotometrically at 421 nm. A standard curve for L-rhamnose was plotted with concentrations ranging from 1 to 200 µg mL^{-1}. Given that rhamnose constitutes a fraction of the rhamnolipid structure, the measured rhamnose mass was multiplied by a correction factor of 3.2 to determine the total concentration of rhamnolipids.

To elucidate the structural and functional groups in the biosurfactant, Fourier Transform Infrared (FT-IR) spectroscopy was performed on a Shimadzu Affinity 1 spectrometer. Sample preparation involved pulverizing the biosurfactant in a ball mill (BM500, Anton Paar), mixing it with potassium bromide, and then pressing the

mixture into pellets. FT-IR spectra were recorded in the wavenumber range of 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}, with 40 scans per sample, and analyzed using OriginPro 8.0 software.

Emulsifying activity was determined by mixing 2 mL of the biosurfactant with an equal volume of commercial mineral oil (Cimed, Marabá, Brazil). The mixture was agitated vigorously for two minutes and allowed to rest for 24 hours, from which the emulsification index (EI24%) was calculated following the methodology of Santos *et al*. (2024).

An evaluation of the chemical activity of the biosurfactant in aqueous solutions indicates the concentration at which maximum solubility and bioavailability are achieved. The biosurfactant was completely diluted to a working concentration of 2 mg L^{-1} to 0.5 mg L^{-1}. The surfactant solubility was measured by titration using methyl trityl ammonium bromide, a reagent that can form insoluble complexes with anionic biosurfactants, facilitating titration. Based on the volume of titrant used at the equivalence point and its concentration, the amount of neutralized biosurfactant and, consequently, its solubility were calculated. The solubility of sodium dodecyl sulfate (SDS) was used as a known standard.

Acute toxicity assays

For the acute toxicity assays, a cohort of 20 individuals from a single spawning event, specifically at developmental stage 25 was utilized. Stage 25 of the Gosner (1960) scale – the standard for anurans – represents a pivotal phase in tadpole metamorphosis. During this stage, notable morphological features are evident: hind limb development reaches completion, characterized by elongation, while forelimbs are still in the early stages, typically appearing as small buds at their respective bases. The head becomes distinctly separated from the trunk, with a slight reduction in tail size beginning to occur. Concurrently, solid food intake is enhanced, indicating a transition in dietary habits. The eyes are pronounced, positioned laterally, and feature well-defined irises. Additionally, tail reabsorption progresses, resulting in a smaller tail proportion relative to body size compared to earlier stages. Respiratory changes are also pronounced; external gill structures may still be present or approaching reabsorption, as pulmonary respiration begins to either supplement or fully replace gill-based respiration.

These individuals were distributed across two aquariums, each containing 0.5 liters of dechlorinated water and housing 10 individuals per aquarium. The properties and constituent compounds of this water were analyzed and are presented in Table 1. A completely randomized experimental design was employed, encompassing two treatments: a) the treatment group exposed to a 0.5 mg mL^{-1} biosurfactant suspension in dechlorinated water, and b) a negative control group consisting solely of dechlorinated water. The defined concentration of 0.5 mg L^{-1} is established in accordance with Brazilian Environmental Council (CONAMA), Resolution No. 357/2005 (Brasil, 2005), which outlines criteria for the classification of aquatic ecosystems, establishes quality standards for permissible levels of chemical contaminants, including surfactants.

The individuals were monitored post-exposure for 96 hours, with behavioral observations recorded at four-hour intervals. At the conclusion of the 96-hour exposure, individuals were anesthetized using lidocaine 2% (m/v) (DLA Pharma, São Paulo, Brazil), euthanized, and preserved in Falcon tubes containing a glutaraldehyde 3% (m/v) fixative solution (Êxodo Cientifica, São Paulo, Brazil) to maintain the integrity of the biological material while minimizing alterations to cellular structures. The individuals were subsequently stored until histological section preparation.

Morphological observations included total, caudal, and head lengths, measured using a digital caliper (Series 500, Mitutoyo, Kawasaki, Japan). Histopathological evaluations involved preparing histological sections were prepared using historesin (methacrylate glycol) and sectioned at 3 μm on a Leica RM2245 microtome (Wetzlar, Germany) with glass knives. Following inclusion, cutting, and staining processes with eosin, hematoxylin, and toluidine, slides were analyzed utilizing a light microscope (Nikon Eclipse E200, Tokyo, Japan, 10 × and 40 × objective lenses). All reagents in this section were purchased commercially from Êxodo Cientifica.

Table 1. Physicochemical properties measured for collected water and water with biosurfactant.

Water	pH	NH^2/NH^{4+} (mg L^{-1})	BOD (mg L^{-1})	COD (mg L^{-1})	Turbidity (uT)
Collected Water	6.6 ± 0.2	1.3 ± 0.4	3.4 ± 0.2	5.0± 0.3	0.2
Experiment water	6.1 ± 0.3	1.1 ± 0.1	1.2 ± 0.1	4.5 ± 0.5	0.2
Experiment water with surfactant (0.5 mg L^{-1})	6.0 ± 0.1	1.5 ± 0.3	2.0 ± 0.2	6.1 ± 0.2	0.4

Note: NH^2/NH^{4+}: Ammoniacal nitrogen; BOD: Biochemical oxygen demand; COD: Chemical oxygen demand.

RESULTS AND DISCUSSION

The bacterium *Pseudomonas aeruginosa* BM02 has demonstrated effective biosurfactant production using andiroba waste as the sole nutrient source, as illustrated in Figure 2. The fermentation process was monitored for 19 days, with stabilization of optical density observed by day 13, coinciding with a peak in biosurfactant production (Figure 2A).

Biosurfactant production was assessed through optical density measurements, which serve as an indirect measure of turbidity of bacterial cultures and correlate directly with bacterial cell concentration. As the bacterial population proliferates, a corresponding increase in optical density is noted, with the exponential growth phase representing the period of maximal biosurfactant synthesis due to heightened metabolic activity.

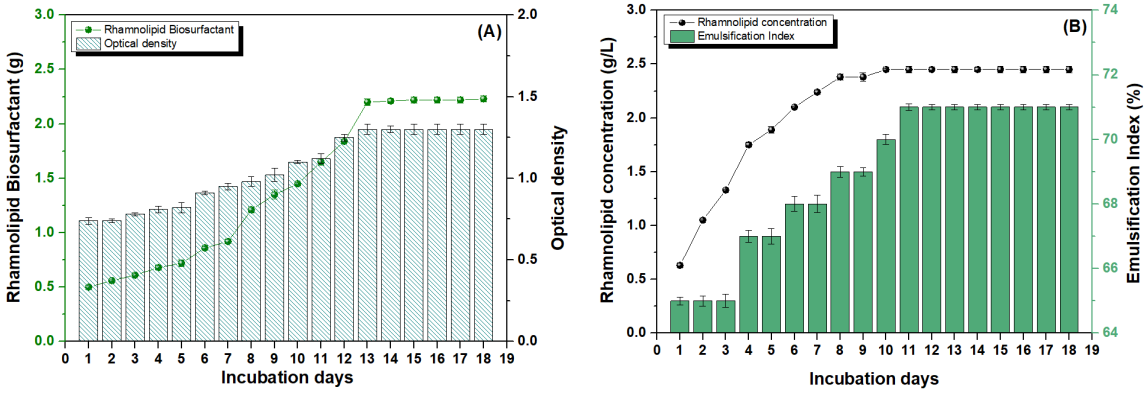

Figure 2. Biosurfactant production by *Pseudomonas aeruginosa* BM02 utilizing andiroba (*Carapa guianensis* Aubl.) waste. **(A)** Correlation between extracted biosurfactant mass (expressed in g) and the emulsification index, measured by optical density throughout the incubation period, and (B) relationship between rhamnolipid concentration (expressed in g L^{-1}) and emulsification index throughout incubation.

The established relationship between biosurfactant production and bacterial growth phases, particularly during the exponential phase, highlights the utility of optical density as an indirect indicator of biosurfactant synthesis (Pinto *et al*., 2009). By correlating optical density with biosurfactant yields, optimization of culture conditions for enhanced production becomes feasible (Souza *et al*., 2025). Bacterial growth curves, represented through optical density over time, are effective for monitoring biosurfactant synthesis (Santos, 2019).

As indicated in Figure 2B, the biosurfactant yield was approximately 2.5 g L^{-1}, comparable to yields obtained using glycerol as substrate with the same strain (Santos *et al*., 2024). This peak yield was achieved and stabilized by the 11th day of production, coinciding with maximum emulsifying activity of around 72%. In contrast, biosurfactants produced by *P. aeruginosa* GB-3 utilizing synthetic substrates resulted in a yield of only 0.15 g L^{-1} (Zhu *et al*., 2024). This comparative analysis highlights the superior biosurfactant production capability of *P. aeruginosa* BM02, particularly when utilizing non-conventional substrates like andiroba waste. This finding emphasizes the effective relationship between biosurfactant concentration and emulsifying activity.

The bacterium *P. aeruginosa* is well-documented for producing rhamnolipid biosurfactants, predominantly consisting of rhamnose (Wittgens & Rosenau, 2020), a unique six-carbon “deoxy” sugar lacking an oxygen atom (Cheng *et al*., 2017). Various factors, including nutrient availability, microbial growth phase, and emulsifying activity, can significantly influence rhamnose production stabilization. Increased rhamnose concentrations in rhamnolipids have been shown to enhance the emulsification index of biosurfactants, as rhamnose facilitates micelle formation, leading to improved emulsion stability over extended periods (Kaskatepe & Yildiz, 2016). Collectively, these findings indicate that the biosurfactant extraction conditions are favorable for achieving both high yields and effective emulsion stabilization. Additional experimental data elucidating the influence of nutrient ratios on biosurfactant yield and emulsification performance would reinforce these observations.

The ecological implications of utilizing andiroba waste as a nutrient source for biosurfactant production are profound, especially from a sustainability and waste valorization perspective. The transformation of agricultural or industrial waste into valuable products, such as biosurfactants, promotes a circular economy and mitigates environmental pollution associated with waste accumulation (Koul *et al*., 2022; Sá *et al*., 2025). By utilizing frequently discarded andiroba waste, this research promotes the sustainable utilization of natural resources and advocates for the practical application of waste materials in bioprocesses (Koul *et al*., 2022). Furthermore, the produced biosurfactants have diverse applications, including bioremediation, oil spill management, and various industrial processes (de Almeida Alves *et al*., 2025; Souza *et al*., 2025), underscoring their role in fostering ecological sustainability.

The structural characterization of the biosurfactant was performed using Fourier Transform Infrared Spectroscopy (FT-IR), with the resulting spectra depicted in Figure 3A. The spectral analysis revealed a prominent band at 3310 cm^{-1}, attributed to the stretching vibrations of hydroxyl groups (O-H) linked to rhamnose (Silva *et al*., 2023). Bands at 2928 cm^{-1} and 2847 cm^{-1} indicated the presence of the methylene and methyl group stretching vibrations, while a significant band at 1633 cm^{-1} confirmed the existence of carbonyl (C=O) groups derived from fatty acids (Guzella *et al.*, 2022; Silva *et al*., 2023). The bands at 1444 cm^{-1} and 1089 cm^{-1} correspond to ether bonds (C-O-C) within the rhamnolipid structure, while bands below 1000 cm^{-1} correlated with the glycolipid component of the biosurfactant. Collectively, these findings affirm that the biosurfactant exhibits a chemical structure typical of rhamnolipids.

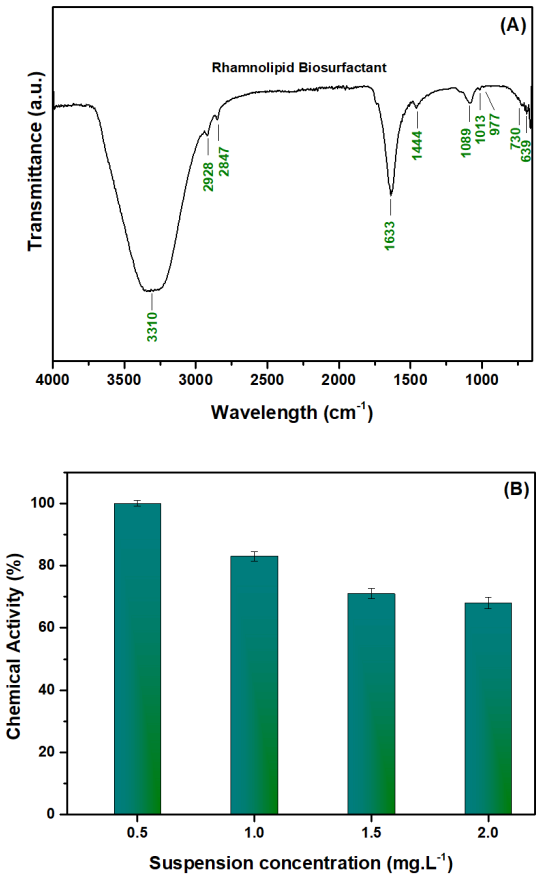

Figure 3. Structural characterization and water solubility of the biosurfactant. **(A)** FT-IR analysis of the extracted biosurfactant, and **(B)** correlation between biosurfactant concentration (expressed in mg L^{-1}) and its chemical activity in aqueous solution (expressed in %).

The balanced arrangement of hydrophilic and hydrophobic moieties in the rhamnolipid structure, as revealed by FT-IR analysis, suggests its low toxicity and potential biodegradability (Sharma & Banerjee, 2024). This characteristic indicates the likelihood of rapid delineates the criteria for water body classification and sets quality standards for chemical substances, including environmental degradation, thus minimizing the risks of accumulation and adverse long-term ecotoxicological effects, particularly given the established low toxicity profile of rhamnolipids.

Water solubility is a crucial parameter influencing the biosurfactant's interaction with aquatic organisms, ultimately determining its bioavailability (Kaczorek *et al.*, 2018). As detailed in Figure 3B, the biosurfactant's chemical activity in aqueous solutions indicates the concentration at which maximum solubility and bioavailability are attained. The biosurfactant was completely diluted to a working concentration of 0.5 mg L^{-1}, consistent with the experimental design. Water-soluble biosurfactants are known to have a pronounced effect on aquatic organisms, potentially mitigating long-term deleterious impacts on biological communities (Karmakar *et al*., 2025). However, results indicate that concentrations exceeding 0.5 mg L^{-1} may reduce the biosurfactant's chemical activity, likely due to the formation of aggregates at higher concentrations, which may hinder dissolution efficacy.

Water quality is a determining factor of aquatic ecosystem balance and the health of resident organisms. In the present study, several physical and chemical parameters of water were examined, including pH, ammoniacal nitrogen, BOD, COD, and turbidity, to assess the environmental conditions' suitability for tadpoles. The findings are summarized in Table 1.

The data indicated that water from different groups – including the original habitat water, experimental water, and biosurfactant-containing water – exhibited values within established environmental quality standards, ensuring the viability of the tested organisms. The pH of the water remained within the acceptable range for aquatic life (6-9), avoiding extreme variations that could compromise tadpole survival (APHA, 2005). Ammoniacal nitrogen levels also remained within acceptable limits (<1.5 mg L^{-1}) for regimentations (APHA, 2005), avoiding harmful concentrations impacting organism metabolism. Furthermore, BOD (<5 mg L^{-1}) and COD (<20 mg L^{-1}) analyses revealed values compatible with healthy environments and indicating a low presence of organic and inorganic contaminants that could deplete dissolved oxygen levels. Turbidity remained low, ensuring adequate light penetration and respiration conditions for the tadpoles. Validating these parameters within environmental standards is crucial for conducting reliable cytotoxicity assessments of the biosurfactant.

Conducting toxicity evaluations under CONAMA Resolution No. 357/2005 (Brasil, 2005) is crucial for ensuring adherence to established protocols to protect environmental and public health. This resolution surfactants. Although it does not specify an upper limit for surfactant concentrations in water, it establishes

comprehensive standards for water quality and effluent discharge to mitigate harmful contamination risks. Notably, concentrations of commercial surfactants exceeding 0.5 mg L^{-1} in aquatic environments could pose potential risks to surrounding communities. Therefore, assessing the obtained biosurfactant under similar regulatory frameworks as commercial surfactants is essential to facilitate a robust cost-benefit analysis and establish a foundation for assessing environmental compatibility.

The findings indicate that tadpoles exposed to a biosurfactant concentration of 0.5 mg L^{-1} exhibited a slight reduction in morphological parameters compared to the control cohort (Figure 4). The control tadpoles (Figure 4A) measured a total length of 17.87 ± 0.94 mm, while tadpoles exposed to the biosurfactant (Figure 4B) averaged 15.30 ± 1.15 mm. A modest decrease in head and tail lengths was observed, decreasing from 3.17 ± 0.34 mm to 2.81 ± 0.56 mm and 12.84 ± 0.60 mm to 10.62 ± 1.42 mm, respectively. These results suggest that exposure to biosurfactant could influence tadpole morphology, resulting in subtle dimensional changes.

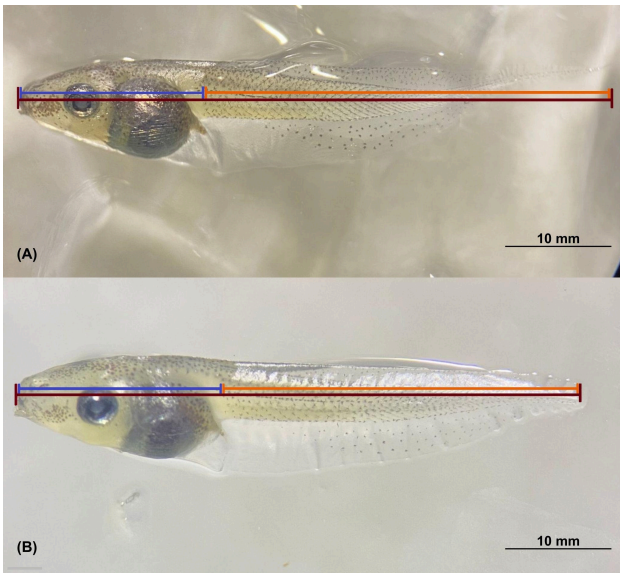

Figure 4. Morphological parameters of *Pithecopus hypochondrialis* tadpoles. **(A)** Tadpoles exposed to dechlorinated water, and **(B)** tadpoles exposed to a 0.5 mg L^{-1} biosurfactant solution. Morphological mensuration included total (colored in brown), caudal (colored in orange), and head (colored in blue) lengths.

Importantly, the observed morphological variations do not necessarily indicate direct toxicity; they may represent physiological adaptations to the biosurfactant, a response frequently noted in organisms exposed to exogenous compounds (Trudeau *et al*., 2020). While the reductions in total length, head, and tail size suggest alterations in growth patterns, it is noteworthy that tadpoles maintained consistent behavioral and ecological traits throughout the experimental period. The absence of significant behavioral modifications, such as impairments in foraging and swimming, indicates that the biosurfactant exposure does not adversely affect the overall health of the organisms, suggesting safety in this experimental context.

Furthermore, the reductions in morphology could reflect adaptive responses to environmental conditions or intrinsic alterations in growth rates or metabolic processes, as discussed by Stehouwer (1992). Thus, the dimensional reductions may indicate organizational responses to microhabitat variability rather than health decline. Investigating these adaptive mechanisms could provide valuable insights into the tadpole responses to various environmental challenges and chemical agents, including biosurfactants.

Zicarelli *et al*. (2024) and Xu *et al*. (2019) demonstrate that exposure to commercial chemical surfactants at concentrations ranging from 0.1 to 100 mg L^{-1} resulted in various morphological impairments in the African clawed frog, *Xenopus laevis*, including spinal and tail deformities, increased pigmentation, and disrupted metamorphosis. Additional morphological and physiological impairments of surfactant exposure in amphibians, along with implications for other terrestrial and aquatic organisms, are summarized in Table 2. These studies underscore the potential for surfactant exposure to induce direct morphological alterations and carry broader ecological consequences, such as altered predator-prey dynamics influenced by modified mobility and visibility.

Histopathological evaluations of tadpoles exposed to the biosurfactant indicated no signs of acute toxicity (Figure 5). Histological assessments revealed well-organized epithelial tissue, indicative of intact cellular structure and function. Muscle tissue remained normal, without any signs of degeneration or necrosis, and cartilaginous tissue along with chondrocyte morphology appeared preserved, showing no lesions or abnormalities. The arrangement of columnar cells was well-defined, showing no evidence of hyperplasia or dysplasia. Nuclear morphology remained normal, with functional nuclei devoid of apoptosis or necrosis.

Given that amphibian skin serves as a critical innate immune barrier and a primary defense against environmental pathogens (Varga *et al*., 2019), the integrity of epithelial and muscular cells implies that the structural viability of the tadpoles was not compromised by biosurfactant exposure. The absence of hyperplasia in intestinal columnar cells further suggests preserved digestive functionality, with no acute inflammatory responses evident in the gut environment. Pryor (2014) highlights the importance of maintaining intestinal integrity in anuran tadpole populations for successful captive breeding programs. Overall, these findings indicate that exposure to 0.5 mg L^{-1} of the biosurfactant does not induce significant adverse effects on tadpole survival, suggesting its toxicological safety within the study's framework.

Table 2. Documented toxicity of chemical surfactants across various organisms.

Organism	Toxic response	Concentration (mg L^{-1})	Reference
African clawed frog *Xenopus laevis*	Spinal and tail deformities, edema, increased pigmentation, and full body deformities	0.1 – 100	Zicarelli *et al.* (2024)
	Metamorphosis alterations driven by thyroid hormones	10.13 – 59.14	Xu *et al*. (2019)
Salamanders *Ambystoma* sp.	Genotoxic damage, including micronucleus	0.258 – 112.5	Zavala-Aguirre *et al*. (2007)
Microcrustacean *Daphnia magna*	Ciliary activity	6.0 – 7.0	Lagerspetz *et al*. (1993)
Whitefish *Coregonus clupeaformis*	Olfactory bulbar electrical responses to odorants	0.1	Hara & Thompson (1978)
Microcrustacean *Ceriodaphnia dubia*	Lethality	1.26	Harmon *et al.* (2003)
Horn sharks *Heterodontus francisci*	Repellency	43.6	Smith (1991)
Topsmelt *Atherinops affinis*	Compromise on growth	0.001 – 0.002	Hemmer *et al*. (1992)

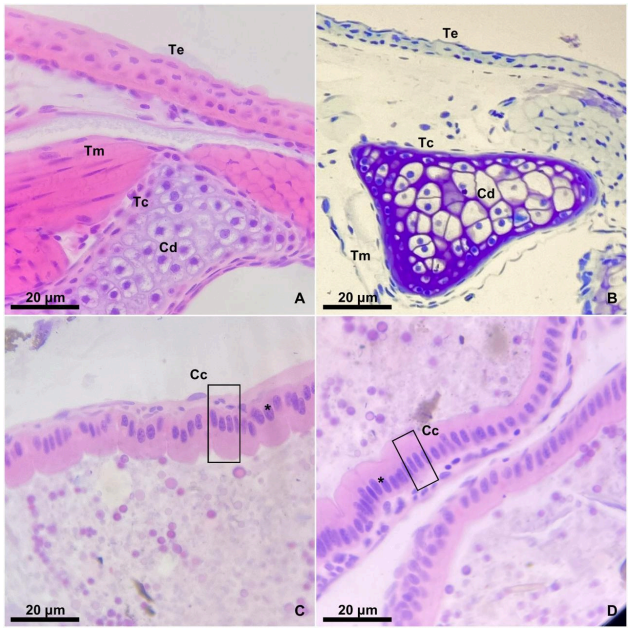

Figure 5. Histopathological examination of the craniofacial region in *Pithecopus hypochondrialis* tadpoles. **(A and C)** Negative control group consisting solely tadpoles exposed to dechlorinated water, and **(B and D)** treatment group consisting tadpoles exposed to a 0.5 mg L^{-1} biosurfactant solution. Histological features include **(Cc)** columnar cells, **(*)** nucleus, **(Te)** epithelial tissue, **(Tc)** cartilage tissue, **(Cd)** chondrocytes, and **(Tm)** muscle tissue.

CONCLUSIONS

The findings presented in this study demonstrate that the bacterium *Pseudomonas aeruginosa* BM02 is highly effective in biosynthesizing rhamnolipid-type biosurfactants from andiroba waste. The yield and emulsifying activity achieved exceeds those obtained through traditional methods and synthetic substrates. This bioprocess not only offers an eco-friendly production method but also enhances the sustainability of the Amazonian fruit production chain, thereby supporting the principles of a circular bioeconomy within the Amazon region through the valorization of plant waste.

Toxicity assessments performed on *Pithecopus hypochondrialis* tadpoles revealed that exposure to the biosurfactant at concentration of 0.5 mg L^{-1} did not result in significant adverse effects, suggesting a level of safety for the biosurfactant under the investigated conditions. These findings open avenues for potential applications where non-toxic compounds are prioritized.

Future investigations should focus on exploring the multi-generational and long-term impacts of biosurfactant exposure on tadpole development and adult fitness. These studies are critical for understanding the implications of subtle morphological changes observed,

particularly regarding their potential impacts on reproductive success and the viability of subsequent generations. Additionally, behavioral analyses across varying exposure scenarios may clarify the interplay between morphological alterations and functional fitness. A systematic examination of the biosurfactant impacts across various amphibian species and developmental stages will enhance the understanding of their ecological consequences. Until such data are available, caution should be advised in its widespread use.

To improve the robustness and applicability of future inquiries, researchers should adopt a multi-dimensional approach that investigates the biochemical and ecological interactions of biosurfactants in diverse environmental contexts. Encouraging collaborations among microbiologists, ecotoxicologists, and environmental engineers will refine the practical applications of biosurfactants, ensuring alignment with sustainable development initiatives. This integrated effort can significantly advance the ecotoxicology, ensuring that innovations are grounded in solid science and applicable to real-world scenarios.

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CREDIT AUTHOR STATEMENT

JAA: Data curation, Formal analysis, Investigation. **GCSS:** Data curation, Formal analysis, Writing – review & editing. **EOR:** Formal analysis, Investigation. **ESNA:** Formal analysis, Investigation. **DHSS:** Data curation, Formal analysis. **ECS:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **SCS:** Conceptualization, Methodology, Supervision, Resources, Validation, Visualization, Writing – review & editing.

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