Biopolymer production by rhizobacteria associated with Cactaceae

Leonardo Figueiredo Landim[1], Aline Simões da Rocha Bispo[2], Caliane da Silva Braulio[3], Adailson Feitoza de Jesus Santos[4]*

[1] Universidade Estadual da Bahia – UNEB. Departamento de Tecnologia e Ciências Sociais - Campus III. R. Edgar Chastinet, s/n - São Geraldo, Juazeiro - BA, 48905-680. Brazil. E-mail: landim598@gmail.com

[2] Embrapa Mandioca e Fruticultura – EMBRAPA. Rua Embrapa, s/n, Chapadinha, Cruz das Almas – BA, 44380-000. Brazil. Orcid: https://orcid.org/0000-0002-7672-2582. E-mail: alinesimoesbispo@gmail.com.

[3] Universidade Estadual da Bahia – UNEB. Universidade do Estado da Bahia - Campus VIII - Caminho das Águas - Rua da Gangorra, 503 - General Dutra, Paulo Afonso – BA, 48608-240. Orcid: https://orcid.org/0000-0003-3074-2876. E-mail: caliane.braulio@gmail.com

[4] *Universidade Estadual da Bahia – UNEB. Universidade do Estado da Bahia - Campus VIII - Caminho das Águas - Rua da Gangorra, 503 - General Dutra, Paulo Afonso – BA, 48608-240. Orcid: https://orcid.org/0000-0002-7860-7617. E-mail: afsantos@uneb.br. *Autor correspondente

Received in: 16/07/2025
Accepted in: 04/10/2025

Abstract

Bacterial biopolymers are biodegradable substances and sustainable alternatives to conventional polymers. Thus, they have been gaining prominence as sustainable alternatives to petroleum-derived polymers, promoting research aimed at optimizing their production and application in various industrial sectors. In this context, this study aims to select rhizobacteria from the *Caatinga* with the potential to synthesize exopolysaccharides (EPS) and define the optimal conditions for their production. Bacterial suspensions were inoculated on sterile filter paper discs on culture medium inducing EPS production. EPS production was determined by the formation of a mucoid layer and confirmed in the presence of absolute ethanol. The isolates that accumulated the highest amount of EPS were subjected to tests based on the Rotational Central Composite Design (RCCD), varying pH, temperature, and glucose as a carbon source. EPS recovery was evaluated in two treatments: static fermentation and under constant agitation. Among the 15 isolates selected, PH9.1 had the best performance in assay 13 (pH 7.0; temperature 38.5°C; glucose 1 %), with a mucoid layer diameter of 2.15 cm, higher than the others. Moreover, static fermentation resulted in a higher yield of fresh (0.64 g) and dry (0.42 g) mass, standing out as the most efficient condition, which reinforces the biotechnological potential of *Caatinga* rhizobacteria for sustainable EPS production and boosts new industrial applications.

Keywords: Exopolysaccharides. Biofilms. Caatinga. Microbiome. sustainability.

Introduction

Plants adapted to arid and semiarid conditions have a microbiome tolerant to these challenging environments, which can positively influence plant growth and mitigate the adverse effects of abiotic stresses (BEN ZINEB *et al*., 2024). In this context, the bacteria present in the plant microbiome use several mechanisms to survive in semiarid regions, such as the production of biopolymers (exopolysaccharides – EPS), biofilm formation, intracellular accumulation of osmolytes, production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, among others (GROVER *et al*., 2011). Exopolysaccharides, in particular, play an important role in adapting to stress conditions, including saline soils, water stress, and temperature variations (SRIVASTAVA *et al*., 2024).

Furthermore, EPS, also known as biopolymers, have advantages over petroleum-derived polymers due to their biodegradability and lower environmental impact (NAMBIAR *et al*., 2024). In addition to these characteristics, EPS-based products are gaining prominence in the market, as well as having applications in various sectors, such as agriculture, healthcare, food, and construction (HASSANISAADI *et al.*, 2025). Despite the extensive diversity of microbial biopolymers identified in nature, only a few are commercially produced, such as xanthan gum, dextran, and pullulan (MOURO *et al.*, 2024; NAMBIAR *et al.*, 2024).

Although significant advances have been made in the production of microbial EPS and in the optimization of its applications, there are still gaps to be filled, especially regarding the exploration of natural sources for identification of new EPS-producing bacteria and in the definition of optimal substrates to maximize their production (GAN *et al.*, 2024).

Cacti, typical of semiarid environments with high radiation, large thermal amplitudes, low humidity, and poor soils, create in the rhizosphere microenvironments rich in exudates (mucilages, sugars, and organic acids) that favor EPS-producing rhizobacteria (HUANG *et al.*, 1993; FONSECA-GARCÍA *et al*., 2016). In this context, it is important to identify rhizobacteria associated with native cacti that are able to synthesize exopolysaccharides, as well as to determine the ideal conditions for the production of these biopolymers. In this sense, this study aims to select rhizobacteria from the *Caatinga* with the potential to synthesize exopolysaccharides (EPS) and define the optimal conditions for their production.

Material and methods

Isolation of biopolymer-producing rhizobacteria

Bacterial isolates were obtained from samples of rhizosphere, collected at Raso Da Catarina Ecological Station, associated with three plant genera of the Cactaceae family: *Pilosocereus* spp., *Tacinga* spp., and *Melocactus* spp.

For the isolation, the soil fraction firmly adhered to the roots (rhizosphere, 0-10 cm in the root zone) was removed with a sterile spatula and packed in sterile bags, kept in a thermal box (4 °C) until processing (24 h). At the laboratory, 10 g of rhizosphere were transferred to vials containing 90 mL of sterile saline (0.85 % NaCl) with 0.05 % of Tween 80, stirred at 150 rpm for 20-30 min to dislodge bacteria. After decantation for 5 min, the supernatant was subjected to decimal serial dilutions (10^{-1} to 10^{-6}) and 100 μL of each dilution were sown on the surface, by spreading, on nutrient agar/tryptone-soy agar (TSA) and on yeast mannitol agar (YMA), to recover both fast-growing heterotrophs and rhizobacteria favored by mannitol, with screening of mucoid colonies. When necessary, cycloxemide (100 mg L^{-1}) was added to the medium for inhibiting fungi. The plates were incubated at 30 °C for 24-72 h and colonies with distinct morphologies, with attention to mucoid aspects, were successively spiked until pure cultures were obtained.

The pure cultures were kept in corresponding broth and preserved with glycerol (20 %, v/v) at -80 °C, receiving identification codes for use in the subsequent screening steps of EPS production. Negative controls (media without inoculation) were included to verify the asepsis of the procedure. A total of 90 rhizobacteria from the *Caatinga* were obtained.

Selection of EPS producers

The ability of microorganisms to synthesize EPS was evaluated following the methodology described by Paulo *et al*. (2012). For the evaluation, a volume of 5 μL of bacterial suspension (OD600nm = 0.3) was inoculated into sterile filter paper discs (5 mm in diameter) of the bacterial culture standardized at 10^8 CFU mL^{-1} (Colony Forming Unit per milliliter) arranged on modified culture medium, according to Guimarães *et al*. (1999). The composition of the medium included 2 % yeast extract, 1.5 % K_2HPO_4, 0.02 % $MgSO_4$, 0.0015 % $MnSO_4$, 0.0015 % $FeSO_4$, 0.003 % $CaCl_2$, 0.0015 % NaCl, 1.5 % agar, and 10 % sucrose, with pH adjusted to 7.5.

The plates were incubated at 30 ºC for 48 hours and EPS production was evaluated by the absence or presence of mucoid colony around the discs. EPS production was identified by the formation of a mucoid layer around the

filter paper discs. Subsequently, this layer was removed and transferred to test tubes containing 2 mL of absolute ethanol, with the presence of EPS confirmed by the formation of a precipitate as positive and the presence of turbidity as negative (PAULO *et al.*, 2012).

The 15 selected strains were evaluated under different cultivation conditions, using the same culture medium of the initial selection step for EPS production. The experiment was carried out in a factorial design, considering three experimental factors: pH, temperature, and carbon source. The optimization of EPS production conditions was conducted by the Rotational Central Composite Design (RCCD), based on a 2^3 plan, which included six tests under axial conditions and repetitions at the central point, totaling 18 tests with coded values.

The isolates that accumulated the highest production of mucoid substance in the initial test were subjected to different culture conditions, following an RCCD, as described by Rodrigues and Iemma (2009). In this experiment, three independent variables were considered: pH, temperature, and glucose concentration as carbon source (Table 1). The dependent variable was expressed by the diameter of the mucoid layer, measured in centimeters.

The modified culture medium was used, according to Guimarães *et al*. (1999), as described and used in the initial selection step of EPS production, with the specific variations of pH, temperature, and glucose concentration according to the indicated levels. The experimental analysis was performed by measuring the diameter of the mucoid layer around the filter paper discs, using a high-precision caliper. The data obtained were subjected to statistical analysis. The means were compared by Tukey's test, at the level of 5 % significance ($p < 0.05$), using the software Statistic 7.0 (STATSOFT, 2004).

EPS production and recovery

The optimal condition for the production of exopolysaccharides (EPS) was employed in a fermentative process aiming at the recovery of the polymer and the determination of its total dry mass. The isolate that achieved the highest EPS production was previously activated in modified solid culture medium, as described by Guimarães *et al*. (1999). After growth in an incubator at 28 ºC for 24 hours, three aliquots of the culture were transferred to two 250 mL Erlenmeyer flasks containing 50 mL of the same medium, adjusted to the best carbon source (%), pH, and temperature identified as ideal for production, starting the pre-inoculum phase.

Two treatments were established: static fermentation and fermentation under constant stirring (120 rpm), both incubated for 24 hours. Then, 10 mL of the pre-inoculum were added to Erlenmeyer flasks containing 100 mL of culture medium and kept under the same conditions. After 72 hours of incubation, the cells were separated from the fermented broth by centrifugation (14,000 rpm, 15 min, 4 ºC) to determine the fresh and dry mass of the bacterial biomass.

The recovered supernatant was mixed with ice-cold absolute ethanol in a ratio of 1:3 (v/v) and subjected to vigorous stirring for

Table 1. Rotational Central Composite Design (RCCD). Variables and their levels used to provide the best cultivation condition of the selected isolates. Paulo Afonso-BA, 2025.

Variable	-1.68	-1	0	+1	+1.68
pH	5.5	6.1	7.0	7.9	8.5
Temperature (°C)	28.0	32.3	38.5	44.8	49.0
Carbon Source (%)	1.0	2.8	5.5	8.2	10.0

Source: authors (2025).

EPS precipitation. The precipitated material was kept at -20 ºC for 24 hours and then centrifuged (14,000 rpm, 15 min, 4 ºC). The supernatant was discarded, and the precipitated EPS was dried in an oven at 50 ºC until reaching constant dry mass.

To determine the optimization of the processes, the response surface methodology was used along with contour lines, allowing the estimation of the ideal conditions for EPS production.

Statistical analysis

All experiments were performed in triplicate (n = 3). The data were subjected to analysis of variance (ANOVA) in SISVAR (UFLA), with comparison of means by the Scott–Knott test at 5 % significance ($\alpha = 0.05$). The ANOVA assumptions were verified by normality of residuals (Shapiro–Wilk) and homogeneity of variances (Bartlett/Levene); when necessary, Box-Cox transformations were applied. For quantitative factors, polynomial regression models (linear and quadratic) were adjusted in SISVAR, selecting the degree of the model by the significance of the coefficients, adjusted R^2, and analysis of residuals.

Rotational central composite cesign (RCCD)

The statistical analysis was performed in the software Statistica 7.0 (STATSOFT, 2004), using the Response Surface Methodology (RSM). For this, a second-order model was adjusted by the Least Squares Method (LSM), followed by analysis of variance (ANOVA) of the model, lack of adjustment test, diagnosis of residuals, and construction of response surfaces and contour plots, aiming at the identification of the optimal region. The significance level adopted was $\alpha = 0.05$ in all tests.

Results and discussion

The study resulted in the selection of rhizobacteria with potential for the production of biopolymers, including the determination of optimal conditions for the production, recovery, and quantification of the dry mass of biopolymers. A total of 90 *Caatinga* rhizobacteria were isolated from rhizosphere. Among them, 20 were able to produce exopolysaccharides (EPS), and 15 strains stood out for their ability to synthesize mucoid layers in 48 hours, as described in Table 2. The most promising strains were isolated from the rhizosphere and belong to the genera *Tacinga* spp., *Melocactus* spp., and *Pilosocereus* spp.

At the conditions tested, isolate PH9.1 stood out in test 13, performed under the conditions of pH 7.0, temperature 38.5 °C, and 1 % glucose (Table 3). In this test, the isolate reached a diameter of 2.15 cm in the mucoid layer formed around the disc, showing a significantly higher production compared to the other isolates evaluated (Table 3). This result highlights the potential of isolate PH9.1 as a

Table 2. Strains selected with greater emphasis on the production of exopolysaccharides. Paulo Afonso-BA, 2025.

***Tacinga* spp.**	***Melocactus* spp.**	***Pilosocereus* spp.**
T7.1	M7.1	PH9.1
T2.2	M18.1	PH14.1
T7.2	M6.2	PH15.1
T10.2	M7.2	PH16.1
T11.2	M8.2	PH4.2

Source: authors (2025).

Table 3. Factorial planning matrix with coded and real values. Paulo Afonso-BA, 2025.

	pH		Temperature (°C)		Carbon Source (%)		Diameter of mucoid layer (cm)
1	-1	6.1	-1	32.25	-1	2.82	0.66
2	+1	7.9	-1	32.25	-1	2.82	0.92
3	-1	6.1	+1	44.75	-1	2.82	0.97
4	+1	7.9	+1	44.75	-1	2.82	1.69
5	-1	6.1	-1	32.25	+1	8.18	0.21
6	+1	7.9	-1	32.25	+1	8.18	0.35
7	-1	6.1	+1	44.75	+1	8.18	0.71
8	+1	7.9	+1	44.75	+1	8.18	1.38
9	-1.68	5.5	0	38.5	0	5.5	0.75
10	+1.68	8.5	0	38.5	0	5.5	0.69
11	0	7.0	-1.68	28	0	5.5	0.16
12	0	7.0	+1.68	49	0	5.5	1.51
13	0	7.0	0	38.5	-1.68	1	**2.15**
14	0	7.0	0	38.5	+1.68	10	0.37
15	0	7.0	0	38.5	0	5.5	1.18
16	0	7.0	0	38.5	0	5.5	1.46
17	0	7.0	0	38.5	0	5.5	0.52
18	0	7.0	0	38.5	0	5.5	1.35

Source: authors (2025).

promising candidate for EPS production under optimized conditions.

The superior performance of isolate PH9.1 can be attributed to the positive influence of specific growing conditions. The neutral pH (7.0) and temperature of 38.5 °C, close to the optimal limits for many microorganisms, may have favored the metabolic activity of the strain. In addition, the 1 % glucose concentration was sufficient to stimulate EPS synthesis, indicating that higher glucose levels are not necessarily advantageous, possibly due to the specific metabolic efficiency of the isolate.

The larger diameter of the mucoid layer formed around the disc by isolate PH9.1 evidences its high capacity to synthesize EPS under optimized conditions. Such a characteristic positions it as an alternative for biotechnological applications, including the production of biofilms, food stabilizers, and encapsulating agents (MOURO *et al*., 2024; HASSANISAAADI *et al*., 2025).

On the other hand, the differences in performance observed between isolates reinforce the importance of a detailed characterization of the specific conditions that maximize EPS production in each strain (Table 3). These variations can be explained by the genetic and metabolic diversity of rhizobacteria isolated from the *Caatinga* biome (SANTOS *et al*., 2020; DIAS *et al*., 2024), which has unique characteristics, with possibilities of application as biostimulants in cultivated crops and still little explored in terms of Biotechnology.

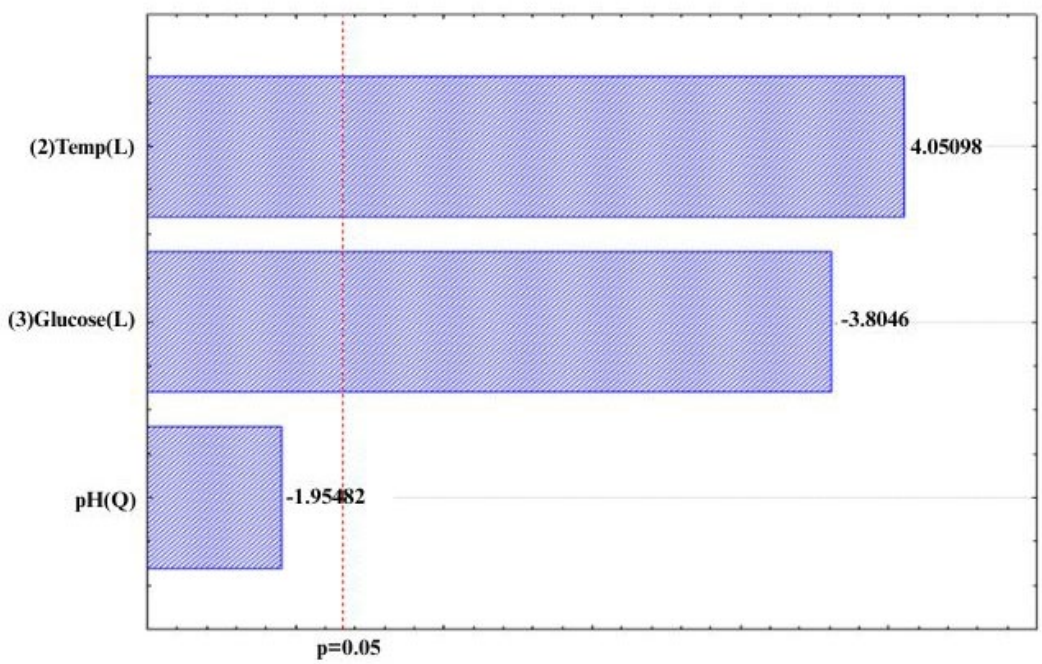
It was verified that the temperature and the concentration of the carbon source (glucose) were the factors that exerted a significant influence ($p < 0.05$) on the production of the polymer under the experimental conditions evaluated (Figure 1). The significant influence

of temperature and glucose concentration on polymer production reflects the crucial role of these variables in microbial metabolism. Temperature is one of the most determining environmental factors for enzymatic activity and, consequently, for the metabolic processes responsible for the synthesis of exopolysaccharides (EPS). Optimal temperatures promote the efficient functioning of enzymes involved in the metabolic pathway of EPS production (WU *et al*., 2025), while deviations to high values can reduce the synthesis rate or induce energy consumption for thermal stress mechanisms.

Glucose concentration, in turn, represents the main source of carbon and energy for the microorganisms tested. Adequate amounts of glucose provide the necessary precursors for EPS biosynthesis (LOOIJESTEIJN *et al*., 1999), while very low concentrations can limit cell growth and polymer production. On the other hand, excessive concentrations can cause the Crabtree effect in some strains, redirecting metabolism to fermentation (SERIKOV *et al*., 2024), which can reduce efficiency in EPS production. Thus, by the result found in this study, the need to optimize the proportion of glucose in the culture medium to maximize the yield of the polymer stands out.

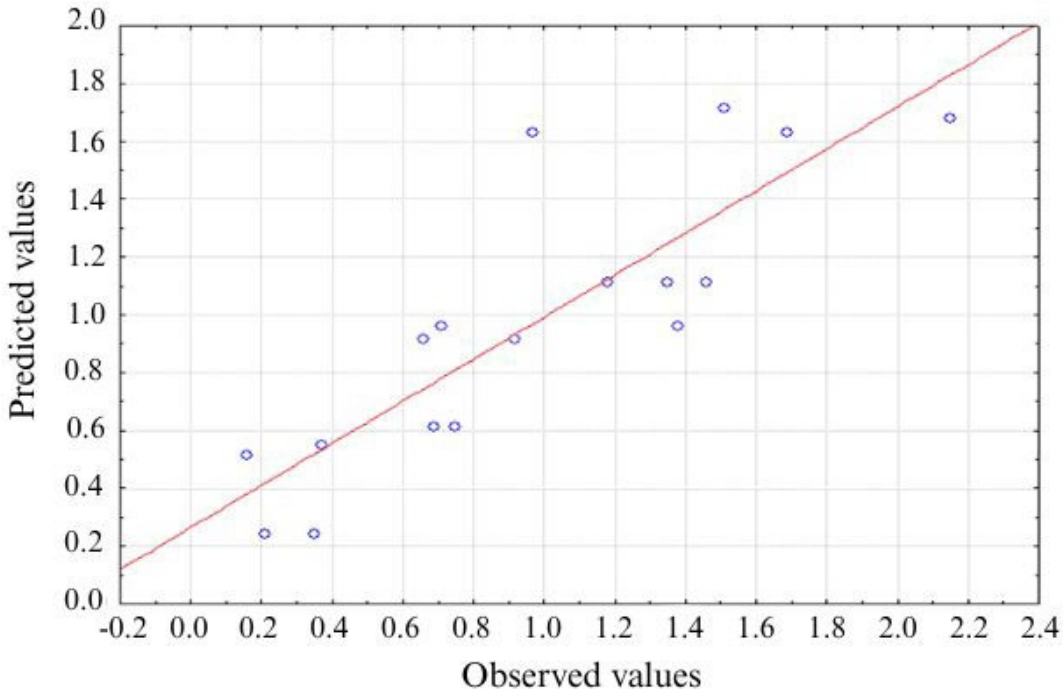
In the analyses performed, linear regression was applied as a statistical tool to identify the most efficient tests. This approach allowed us to obtain a regression that minimized the distance between the experimental points and the proposed model (Figure 2). Based on the results, test 13, characterized by conditions of pH 7.0, temperature of 38.5 °C, and glucose at 1 %, stood out by presenting values closer to the ideal conditions recommended by the model, i.e., 1. We understand that test 13 reached the balance between the variables tested, maximizing polymer production under the conditions evaluated. Therefore, we emphasize the importance of the synergistic interaction between pH, temperature, and glucose concentration (CHAROENWONGPAIBOON

Figure 1. Pareto chart with the relationships between the analyzed variables, with a significance level of 95 %. Paulo Afonso-BA, 2025.



Source: authors (2025).

Figure 2. Values predicted and observed by the model. Blue dots represent trials. The curve indicates the optimal conditions. Paulo Afonso-BA, 2025.



Source: authors (2025).

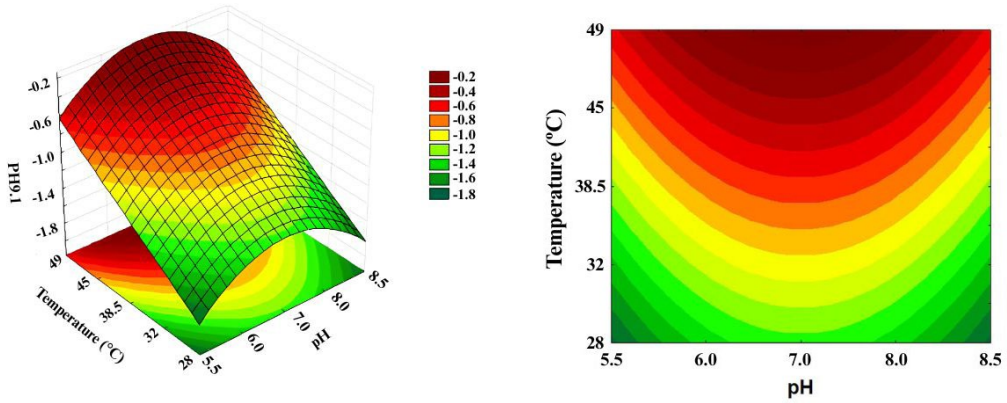
et al., 2024). Moreover, we also highlight the relevance of using regression methods to predict ideal experimental conditions (GUIMARÃES *et al.*, 2020) and potentially increase production efficiency in biotechnological applications.

It was observed that EPS production is non-existent and its specific productivity is not predicted when the temperature is below 30 °C and the pH is below 6.0 or above 8.5 (Figure 3). The maximum production was observed within the range of pH 7.0 and temperatures between 38.5 °C and 49 °C. In the model that considered glucose and pH (Figure 4), both factors had a positive impact on the estimate of maximum production, with pH ranging from 6.0 to 8.0 and glucose concentrations between 1 % and 2.8 %. In the analysis involving glucose and temperature (Figure 5), the maximum estimate was reached with temperatures above 38.5 °C and glucose concentration at 1 %, within the conditions considered ideal for EPS production.

The variables evaluated play a determining role in the synthesis of EPS, with emphasis on the positive impact of the interaction between temperature and pH at specific levels. The ideal conditions identified corroborate the literature, which associates the enzymatic stability and metabolic efficiency of microorganisms to the maintenance of specific environmental parameters (MARASCO *et al*., 2023; YIN *et al*., 2024). The influence of glucose as a carbon source also reaffirms its importance in providing energy and metabolic precursors for the production of biopolymers (AL-ANI, KIM, 2025). Thus, this study not only validates the optimized experimental conditions, but also highlights the biotechnological potential of the strains selected for industrial applications.

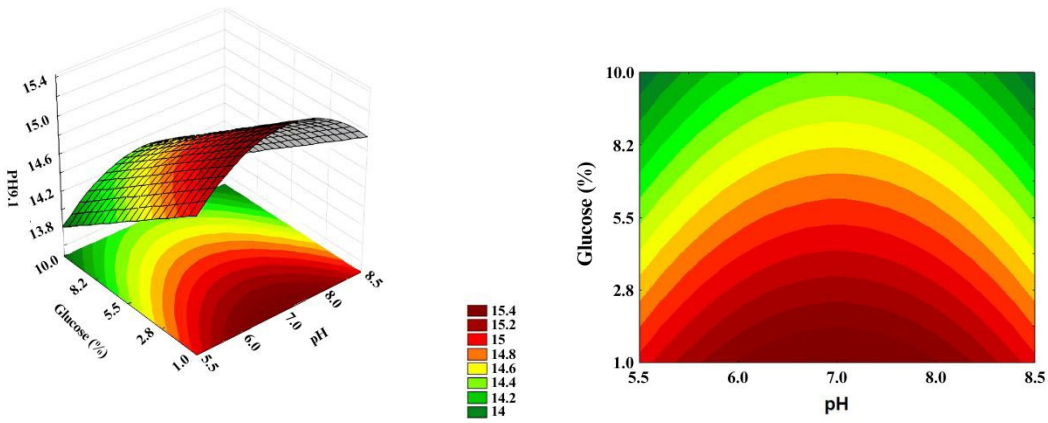
To analyze the procedure used to compare the distribution of three variables in independent samples, analysis of variance (ANOVA) was used to summarize the linear regression model

Figure 3. EPS production by isolate PH9.1 analyzed by response surface and contour line as a function of the variables temperature and pH. Paulo Afonso-BA, 2025.



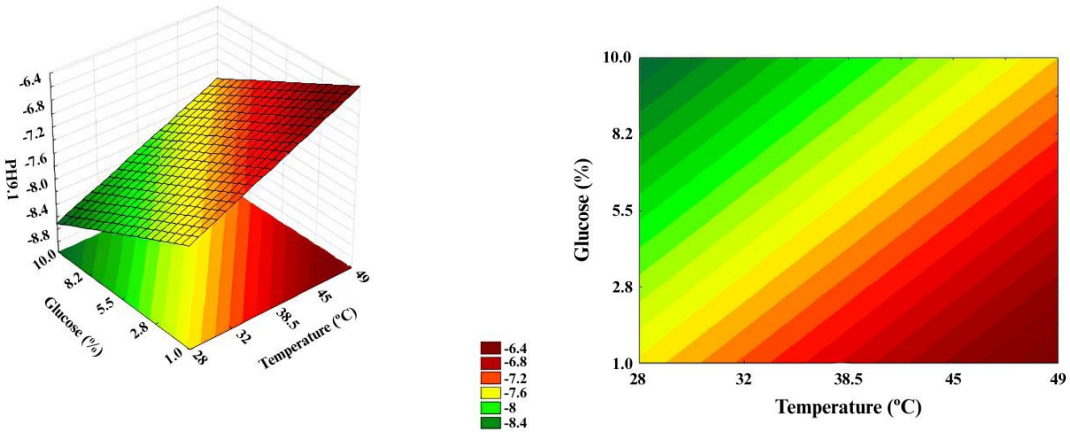
Source: authors (2025).

Figure 4. EPS production by isolate PH9.1 analyzed by response surface and contour line as a function of the variables glucose and pH. Paulo Afonso-BA, 2025.



Source: authors (2025).

Figure 5. EPS production by isolate PH9.1 analyzed by response surface and contour line as a function of the variables glucose and temperature. Paulo Afonso-BA, 2025.



Source: authors (2025).

by each source of variation (Table 4). In this context, high significance was observed for the calculated F value.

In the fermentation step, static fermentation resulted in a thick mucoid layer, pinkish in color, with incrustations on its surface. In contrast, under conditions of constant stirring, no suspended structures were observed, with the medium having a darker coloration. During the biopolymer recovery process, after the addition of ice-cold absolute ethanol as precipitating agent in the volume ratio (supernatant and ethanol), the formation of a mass was observed, indicating the significant presence of EPS. Static fermentation favors the production of EPS with greater morphological visibility (NGUYEN *et al*., 2020), while the recovery of the biopolymer confirms the efficiency of ethanol as a precipitant, making it possible to obtain the biopolymer in large quantities.

The bacterial biomass removed was compared between static and stirred fermentation treatments, and there was no significant difference for the fresh and dry mass of the cells. However, when analyzing the EPS yield after recovery, there was a difference between the two fermentative processes. Fermentation under static conditions achieved higher yield for both fresh and dry EPS mass (Table 5). Although there was no significant variation in bacterial biomass, static fermentation favored a higher production of EPS, which may be related to better synthesis conditions and accumulation of exopolysaccharide under this condition.

Under static fermentation conditions, mixture limitation and shear reduction create local gradients of nutrients and oxygen, modulating the expression of genes related to EPS synthesis (AZIZ, ZAIDI, 2024). This condition can also induce the activation of bacterial stress response systems, such as regulatory systems that adjust EPS production in response to environmental stimuli (CUI *et al*., 2024). Furthermore, static fermentation provides the most favorable environment for the synthesis of biochemical precursors, such as activated sugars (UDP-glucose, GDP-mannose), and for the activity of glycosyltransferases involved in exopolysaccharide polymerization (DEO *et al*., 2024).

Shear reduction, characteristic of this condition, minimizes the degradation of the newly synthesized EPS, promoting its accumulation in the extracellular matrix. These factors highlight the essential role of culture conditions in metabolic targeting, not only for cell growth, but also for the modulation of secondary or extracellular metabolic products, such as EPS. These compounds play crucial roles such as cell protection, biofilm formation, and interaction with the environment, being highly relevant for biotechnological and ecological applications.

Although cellular biomass did not directly influence the final yield under the conditions of test 13, it is evident that, under static fermentation conditions, isolate PH9.1 is influenced by the availability of oxygen in the

Table 4. Analysis of variance (ANOVA) for the analyzed variables. Paulo Afonso-BA, 2025.

Source of variation	SS	DF	MS	Fcalculated
Regression	3.6885	3	1.2295	11.56
Residue	1.3816	13	0.1063	
Total	5.0702	16		

F at 5 %: $F_{0,\ 05}$ (3.13) = 3.41; R^2=73 %. SS: sum of squares, DF: degree of freedom, MS: mean square, F: statistic.

Source: authors (2025).

Table 5. Production of exopolysaccharides (EPS) and cellular biomass of isolate PH9.1 with static fermentation and under stirring. Paulo Afonso-BA, 2025.

Fermentation	FM (cell)	DM (cell)	FM (EPS)	DM (EPS)
Stirring	0.34 a	0.16 a	0.25 b	0.13 b
Static	0.48 a	0.07 a	0.64 a	0.42 a

Means followed by equal letters in the column do not differ from each other by the Scott-Knott test at 5 % probability; FM: fresh mass, DM: dry mass; in mg L^{-1}.

Source: authors (2025).

culture medium for the production of EPS, without necessarily resulting in an increase in microbial biomass. This factor contributes to an increase in the total EPS yield. The results obtained for EPS, both in fresh and dried form, synthesized in static fermentation, were promising. It is inferred that oxygen limitation can act as an essential factor in promoting EPS production, even without a significant expansion of biomass, which is interesting for large-scale biopolymer production processes.

Rhizobacteria from the *Caatinga* can represent a promising source for EPS production, standing out as a sustainable alternative to synthetic polymers derived from petroleum. The exploration of these endemic microorganisms is especially relevant for the development of biotechnological solutions that value the natural resources of the biome, contributing to agricultural sustainability in semiarid regions. In addition to reducing the environmental impacts associated with the disposal of non-biodegradable polymers, the production of EPS by these rhizobacteria can promote more sustainable agricultural practices, helping to conserve soil and strengthen crops that are more resilient to climatic adversities. Therefore, future studies should focus on the structural characterization of EPS to understand its properties and expand its practical applications. Research on the performance of these biopolymers in areas such as agriculture, medicine, cosmetics, and food could consolidate their sustainable use, promoting the regional bioeconomy and conservation of the *Caatinga*.

Conclusions

A total of 15 rhizobacteria from the *Caatinga* with potential for EPS synthesis were selected. Temperature and glucose concentration are the factors that most influence the response, allowing to estimate conditions close to optimal for the evaluated system.

For isolate PH9.1, the best performance was obtained in test 13 (pH 7.0; 38.5 °C; 1 % glucose), with 2.15 cm diameter of the mucoid layer. In the fermentative stage, static fermentation provided a higher EPS yield (fresh and dry mass) without increasing the cellular biomass, being, therefore, the preferred condition for polymer production under the conditions tested.

References

AL-ANI, S.; KIM, Y. Carbon preference by *Cupriavidus necator* for growth and accumulation phases: heterotrophic vs. autotrophic metabolisms. **Journal of Power Sources**, v. 626, p. 235797, 2025. DOI: https://doi.org/10.1016/j.jpowsour.2024.235797

AZIZ, K.; ZAIDI, A. H. Bifidobacterial biofilms as next-generation probiotics and their role in intestinal microbiocenosis. **Critical Reviews in Microbiology**, p. 1-24, 2024. DOI: https://doi.org/10.1080/1040841X.2024.2438119

BEN ZINEB, A.; LAMINE, M.; KHALLEF, A.; HAMDI, H.; AHMED, T.; AL-JABRI, H.;

ALSAFRAN, M.; MLIKI, A.; SAYADI, S.; GARGOURI, M. Harnessing rhizospheric core microbiomes from arid regions for enhancing date palm resilience to climate change effects. **Frontiers in Microbiology**, v. 15, p. 1362722, 2024. DOI: https://doi.org/10.3389/fmicb.2024.1362722

CUI, Y.; WANG, D.; ZHANG, L.; QU, X. Research progress on the regulatory mechanism of biofilm formation in probiotic lactic acid bacteria. **Critical Reviews in Food Science and Nutrition**, v. 65, n. 25, p. 4869-4883, 2024. DOI: https://doi.org/10.1080/10408398.2024.2400593

CHAROENWONGPAIBOON, T.; CHAROENWONGPHAIBUN, C.; WANGPAIBOON, K.; PANPETCH, P.; WANICHACHEVA, N.; PICHYANGKURA, R. Endo-and exo-levanases from *Bacillus subtilis* HM7: catalytic components, synergistic cooperation, and application in fructooligosaccharide synthesis. **International Journal of Biological Macromolecules**, v. 271, p. 132508, 2024. DOI: https://doi.org/10.1016/j.ijbiomac.2024.132508

DEO, R.; LAKRA, U.; OJHA, M.; NIGAM, V. K.; SHARMA, S. R. Exopolysaccharides in microbial interactions: signaling, quorum sensing, and community dynamics. **Natural Product Research**, v. 39, n. 11, p. 1-16, 2024. DOI: https://doi.org/10.1080/14786419.2024.2405867

DIAS, K. C. F. P.; SILVA SOUZA, I. J.: BARROS, Y. C.: SILVA, E. P.: LEITE, J.: FEITOZA, A. F. A.: JESUS SANTOS, A. F. Native bacteria from the caatinga biome mitigate the effects of drought on melon (*Cucumis melo* L.). **Comunicata Scientiae**, v. 15, e4072-e4072, 2024. DOI: https://doi.org/10.14295/cs.v15.4072

FONSECA-GARCÍA, C.; COLEMAN-DERR, D.; GARRIDO, E.; VISEL, A.; TRINGE, S. G.; PARTIDA-MARTÍNEZ, L. P. The cacti microbiome: interplay between habitat-filtering and host-specificity. **Frontiers in Microbiology**, v. 7, n. 150, p. 1-16, 2016. DOI: https://doi.org/10.3389/fmicb.2016.00150

GAN, L.; HUANG, X.; HE, Z.; HE, T. Exopolysaccharide production by salt-tolerant bacteria: recent advances, current challenges, and future prospects. **International Journal of Biological Macromolecules,** v. 264, n. 2, p. 130731, 2024. DOI: https://doi.org/10.1016/j.ijbiomac.2024.130731

GROVER, M.; ALI, S. Z.; SANDHYA, V.; RASUL, A.; VENKATESMARLU, B. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. **World Journal of Microbiology and Biotechnology**, v. 27, p. 1231-1240, 2011. DOI: https://doi.org/10.1007/s11274-010-0572-7

GUIMARÃES, B V. C.; DONATO, S. L. R.; ASPIAZÚ, I.; AZEVEDO, A. N.; CARVALHO, A. J. Regression models for productivity prediction in cactus pear cv. Gigante. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 24, n. 11, p. 721-727, 2020. DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v24n11p721-727

GUIMARÃES, D. P.; COSTA, F, RODRIGUES, M. J.; MAUGERI, F. Optimization of dextran syntesis and acidic hydrolisis by surface response analysis. **Brazilian Journal of Chemical Engineering,** v. 16, p. 129-139, 1999. DOI: https://doi.org/10.1590/S0104-66321999000200004

HASSANISAADI, M.; VATANKHAH, M.; KENNEDY, J. F.; RABIEI, A.; RISEH, R. S. Advancements in xanthan gum: a macromolecule for encapsulating plant probiotic bacteria with enhanced properties. **Carbohydrate Polymers**, v. 348, n. 15, 122801122801, 2025. DOI: https://doi.org/10.1016/j.carbpol.2024.122801

HUANG, B.; NORTH, G. B.; NOBEL, P. S. Soil steaths, photosynthate distribution to roots, and rhizosphere water relations for *Opuntia ficus-indica*. **International Journal of Plant Science,** v. 154, n. 3, p. 425-431, 1993.

LOOIJESTEIJN, P. J.; BOELS, I. C.; KLEEREBEZEM, M.; HUGENHOLTZ, J. Regulation of exopolysaccharide production by *Lactococcus lactis* subsp. *cremoris* by the sugar source. **Applied and Environmental Microbiology**, v. 65, n. 11, p. 5003-5008, 1999. DOI: https://doi.org/10.1128/aem.65.11.5003-5008.1999

MARASCO, R.; FUSI, M.; COSCOLÍN, C.; COSCOLÍN, C.; BAROZZI, A.; ALMENDRAL, D.; BARGIELA, R.; NUTSCHEL, C. G.; PFLEGER, C.; DITTRICH, J.; GOHLKE, H.; MATESANZ, R.; SANCHEZ-CARRILLO, S.; MAPELLI, F.; CHERNIKOVA, T. N.; GOLYSHIN, P. N.; FERRER, M.; DAFFONCHIO, D. Enzyme adaptation to habitat thermal legacy shapes the thermal plasticity of marine microbiomes. **Nature Communcations**, v. 14, n. 1045, 2023. DOI: https://doi.org/10.1038/s41467-023-36610-0

MOURO, C.; GOMES, A. P.; GOUVEIA, I. C. Microbial exopolysaccharides: structure, diversity, applications, and future frontiers in sustainable functional materials. **Polysaccharides**, v. 5, n. 3, p. 241-287, 2024. DOI: https://doi.org/10.3390/polysaccharides5030018

NAMBIAR, K. P, S. K.; DEVARAJ, D.; SEVANAN, M. Development of biopolymers from microbes and their environmental applications. **Physical Sciences Reviews**, v. 9, n. 4, p. 1903-1929, 2024. DOI: https://doi.org/10.1515/psr-2022-0219

NGUYEN, P. T.; NGUYEN, T. T.; BUI, D. C.; HONG, P. T.; HOANG, Q. K.; NGUYEN, H. T. Exopolysaccharide production by lactic acid bacteria: the manipulation of environmental stresses for industrial applications. **AIMS Microbiol**, v. 6, p. 451–469, 2020. DOI: https://doi.org/10.3390/biom14091162

PAULO, E. M.; VASCONCELOS, M. P.; OLIVEIRA, I. S.; AFFE, H. M. J.; NASCIMENTO, R.; MELO I. S.; ROQUE, M. R. A.; ASSIS, S. A. An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with rapid confirmation. **Food Science and Technology**, v. 32, p. 710-714, 2012. DOI: https://doi.org/10.1590/S0101-20612012005000094

RODRIGUES, M. I.; IEMMA, F. A. **Planejamento de experimentos e otimização de processos.** Campinas: Cárita, 2009. 238 p.

SANTOS, A. F. J.; MORAIS, J. S.; MIRANDA, J. S.; MOREIRA, Z. P. M.; FEITOZA, A. F. A.; LEITE, J.; FERNANDES-JÚNIOR, P. I. Cacti-associated rhizobacteria from Brazilian Caatinga biome induce maize growth promotion and alleviate abiotic stress. **Revista Brasileira de Ciências Agrárias**, v. 15, n. 3, e8221, 2020. DOI: https://doi.org/10.5039/agraria.v15i3a8221

SERIKOV, T. A.; JAMALOVA, G. A.; RAFIKOVA, K. S.; K YELIKBAYEV, B.; YERNAZAROVA, A. K.; SERIKOVNA, K. L.; ZAZYBIN, A. G.; SAKHANIN, V. S.; EGUTKIN, V. Y. Ecological, biological and biotechnological aspects of *Saccharomyces cerevisiae* biomass production. **Caspian Journal of Environmental Sciences,** v. 22, n. 2, p. 499-512, 2024. DOI: https://doi.org/10.22124/cjes.2023.7327

SRIVASTAVA, S.; BHATTACHARJEE, A.; DUBEY, S.; SHARMA, S. Bacterial exopolysaccharide amendment improves the shelf life and functional efficacy of bioinoculant under salinity stress. **Journal of Applied Microbiology**, v. 135, n. 7, p. 1-16, 2024. DOI: https://doi.org/10.1093/jambio/lxae166

STATSOFT Inc. **Statistica: data analysis software system, version 7.0.** Tulsa: StatSoft Inc., 2004. Disponível em: http://www.statsoft.com. Acesso em: 1 out. 2025.

YIN, Q.; HE, K.; COLLINS, G.; VRIEZE, J.; WU, G. Microbial strategies driving low concentration substrate degradation for sustainable remediation solutions. **npj Clean Water**, v. 7, n. 1, p. 1-14, 2024. DOI: https://doi.org/10.1038/s41545-024-00348-z

WU, S.; WANG, F.; WANG, H.; SHEN, C.; YU, K. Meta-analysis of abiotic conditions affecting exopolysaccharide production in cyanobacteria. **Metabolites**, v. 15, n. 2, p. 1-13, 2025. DOI: https://doi.org/10.3390/metabo15020131