

## Original Research Article

# Optimization of L-asparaginase activity of Actinobacteria isolated from Guaviare river sediments in Colombia

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## Abstract

**Purpose:** To optimize the L-asparaginase activity of Actinobacteria isolated from Guaviare river sediments in Colombia.

**Methods:** Actinobacterial strains were evaluated for their L-asparaginase activity using phenol red plates and Nessler's assays. Strains with L-asparaginase activity were identified based on 16S ribosomal rRNA sequencing, and a central composite design was used to study nutritional and growth factors that could improve L-asparaginase activity. L-asparaginase protein was detected using western blotting and the cytotoxicity of L-asparaginase preparations was evaluated against MDA-MB231 and L929 cell lines.

**Results:** *Kitasatospora atroaurantiaca*, *Streptomyces griseoluteus*, and *Streptomyces panaciradicis* were cultured in medium with lactose as a carbon source and a combination of asparagine and malt extract as nitrogen sources. These strains showed L-asparaginase activities of 29.4, 114.06, and 34.08 U/mg, respectively, and half-maximal inhibitory concentration ( $IC_{50}$ ) values of  $25.61 \pm 2.15$ ,  $8.18 \pm 1.61$ , and  $165.29 \pm 1.06$  ppm, respectively, against MDA-MB 231 cells. Western blotting analysis revealed the presence of an L-asparaginase monomer with a molecular weight of 37 kDa.

**Conclusion:** *Kitasatospora atroaurantiaca*, *Streptomyces griseoluteus*, and *Streptomyces Panaciradicis* produce L-asparaginases with low L-glutaminase activity and promising cytotoxic activity and thus may be useful for the management of acute lymphoblastic leukemia.

**Keywords:** L-asparaginase, *Streptomyces*, *Kitasatospora*, Optimization, Cytotoxicity

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## INTRODUCTION

L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) is a potential anti-cancer enzyme. This enzyme decreases the L-asparagine concentration by catalyzing the deamination of the amino acid into L-aspartic acid and

ammonium, leading to cell death [1]. Patients with acute lymphoblastic leukemia (ALL) are treated with *Escherichia coli* L-asparaginase; however, they can suffer an adverse reaction because of the L-glutaminase activity together with the short half-life of the enzyme [3-4]. Survival in pediatric patients with ALL has

improved significantly: Nearly 90% of children are cured using L-asparaginase [5].

Therefore, there is a high demand for oncolytic enzymes because cancer cells are more sensitive to them [2]. L-asparaginases produced by microorganisms and plants have been studied, and several attempts have been made to increase their activity [9–12] using different experimental designs, such as the Box-Behnken [13] and Plackett-Burman [12] methods. Likewise, attempts have been made to produce enzyme recombinantly, employing techniques such as directed evolution and epitope engineering, to introduce the gene into *E. coli* [8]; however, the recovery of the enzyme was low, it showed poor biochemical properties [6], and was impure [7].

Thus, the present study focused on searching for novel sources of L-asparaginase with low L-glutaminase activity, and the effects of L-asparaginase from *Actinobacteria* isolated from the Guaviare river (Colombia).

## EXPERIMENTAL

### Biological materials

The strains analyzed belonged to the *Actinobacteria* biobank of La Sabana University and were isolated from sediments of the Guaviare river [18].

### Selection of *Actinobacteria* with L-Asparaginase or L-Glutaminase activity

The morphologies of the strains were evaluated on ISP-3 medium (also known as oatmeal agar) using Gram staining and scanning electron microscopy (SEM) (Phenom pro, Thermo Fisher Scientific, Netherlands) [14]. Strains selection was performed using a phenol red plate assay [15,16]. Strains were cultured in ISP-5-agar (glycerol-L-asparagine) with 0.009 % (v/v) phenol red and incubated at 37 °C for 7 days [15].

### Determination of the enzymatic activities

The enzymatic activities were quantified using nesslerization [16]. The strains were grown in ISP-5 medium at 30 °C, with shaking at 150 rpm (Innova 42R, New Brunswick™, USA) for seven days. After centrifugation at 5000 g (Mikro 22R, Hettich, Germany) for 30 min, the biomass was filtered through a 0.22-µm pore size hydrophilic polyvinylidene fluoride (PVDF) membrane, washed and dried at 80 °C for 24 h. The biomass content was measured and the supernatant (5 mL) was subjected to nesslerization [16]. The

enzymatic activity is expressed in enzyme units (U), which were defined as the amount of enzyme used to release 1 µmol of ammonium per unit time ( $U = \mu\text{mol}/\text{min}$ ) [14]. Protein quantification was performed using a bicinchoninic acid (BCA) assay with bovine serum albumin (BSA) at 2 mg/mL as a standard [17].

### Molecular identification of *Actinobacteria* strains

The 16S rRNA gene was amplified using polymerase chain reaction (PCR) in a thermocycler (BIO-RAD iCycler, USA) with extracted DNA as the template [18]. The PCR reaction was confirmed by electrophoresis in 1x Tris-Borate-EDTA (TBE) buffer and compared with a ladder of molecular markers (HyperLadder™ IV, Bioline, London). The amplicons were sequenced by BIOS-SIB (Colombia) and compared with entries in the GenBank databases and evaluated according to their percent identity.

### Western blotting

Protein extracts (30 µg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes using a Semi-Dry Blotter (EU-4000, C.B.S. Scientific Company Inc, USA) for 30 minutes at 100 V. The membranes were blocked with 5% non-fat dry milk-TBST (Tris-buffered saline-Tween 20) buffer for 1 h and incubated overnight at 4 °C with a 1:1000 dilution of anti-L-asparaginase antibodies conjugated with horseradish peroxidase (HRP) (GTX 40848, GeneTex) in 1% non-fat dry milk-TBST buffer. Bands were detected using myECL Imager (Thermo Scientific, USA). A commercial L-asparaginase (Medac GmbH, Hamburg, Germany) was used as the positive control.

### Evaluation of the effect of carbon and nitrogen sources on the L-asparaginase activity

The best three carbon sources were determined among soluble starch, lactose, glycerol, glucose and sucrose [2,10,15,19] at 1 % (w/v) each, using the phenol red plate assay [15, 20]. The best carbon source was then confirmed using nesslerization. The same tests were performed to determine the best nitrogen source among meat extract, yeast extract, potassium nitrate, peptone, malt extract, L-asparagine, and L-glutamine [6,20] at 1% (w/v), and two combinations of L-asparagine with malt extract or potassium nitrate (both at 0.05%).

### Optimization of nutritional and growth factors

The influence of different nutritional and fermentation conditions on the L-asparaginase (Table 2). The experimental results of CCD were fitted by the response surface regression procedure using a second order polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \beta_{ij} x_i x_j + \sum_{i=1}^n \beta_{ii} x_i^2 \dots \dots (1)$$

where  $Y$  is the predicted response,  $\beta_0$  is the regression coefficient,  $\beta_i$  is the linear coefficient,  $\beta_{ii}$  is the quadratic coefficient,  $\beta_{ij}$  is the interaction coefficient, and  $X_i$  is the coded level of the independent variables [12].

### Evaluation of cytotoxic activity

Cell cultures of MDA-MB-231 (ATCC<sup>®</sup> HTB-26<sup>™</sup>-Breast cancer) and L929 (ATCC<sup>®</sup> CCL-1<sup>™</sup>-mouse fibroblasts) were seeded into 96-well plates in 100  $\mu$ L of Dulbecco's modified Eagle's medium (DMEM) and incubated for 24 h at 37 °C and 5 % CO<sub>2</sub>. L-asparaginase preparations were added (10–200 ppm; 0.26–6.14 mU/mL) for 48 h. Cytotoxic activity was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test [21]. L929 cells were used as the untransformed control cell line. Doxorubicin (1–20 ppm) was used as the cytotoxic positive control.

### Statistical analysis

Analysis of variance (ANOVA) was applied to the established regression and was carried out for the experimental designs and the models, with statistical significance defined as  $p < 0.05$ , in Design Experts<sup>®</sup> v7 software. The surface plots were constructed using STATISTICA software.

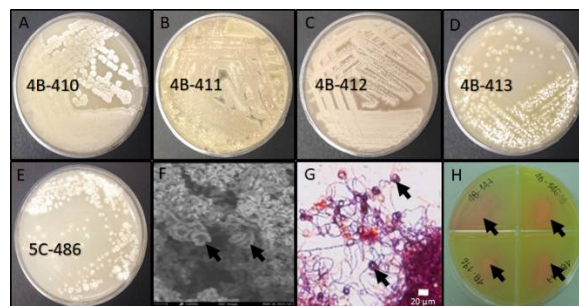
## RESULTS

### Actinobacteria with L-asparaginase and/or L-glutaminase activity

Gram-positive bacteria with the typical morphology of the *Actinobacteria* (Figure 1 A-D) were identified using Gram staining and SEM. *Actinobacteria* showed white aerial and gray vegetative mycelia (Figure 1E), with well-developed and non-fragmented hyphae, with ramifications accompanied by fragments stick or coconut form, the presence of spores in spiral chain on the aerial mycelium (Figure 1F), and the presence of branched thin filaments and spiral structures (

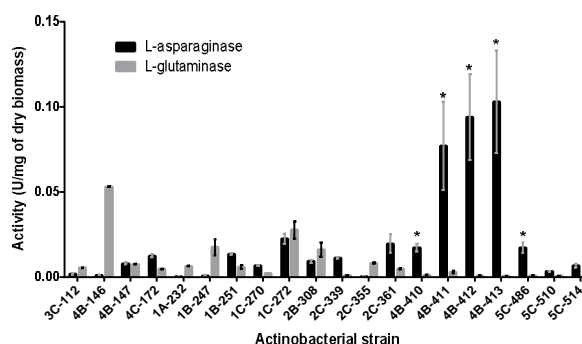
activity was determined using a small central composite design (CCD) with five levels of each factor (

Figure 1G); Among 375 strains tested, 20 presented L-asparaginase and/or L-glutaminase activity, as determined by the phenol red assay [1,10] (Figure 1H).



**Figure 1:** A–E: L-asparaginase producing strains grown on ISP3-agar. F–G: Microscopic features of strain 4B-413 (14000x) observed using scanning electron microscopy (SEM) and Gram staining. Arrows show the presence of spores in spiral chains on aerial mycelium. H: *Actinobacteria* strains identified as positive for the liberation of ammonia by L-asparaginase and/or L-glutaminase activity

Five strains showed higher L-asparaginase than L-glutaminase activity (Figure 2). Strain 4B-413 showed the highest L-asparaginase activity with an L-asparaginase/L-glutaminase ratio of almost 200.



**Figure 2:** L-asparaginase/L-glutaminase activities of 20 actinobacterial strains. The results are shown as mean  $\pm$  SD (n = 3). \*Strains selected for further research

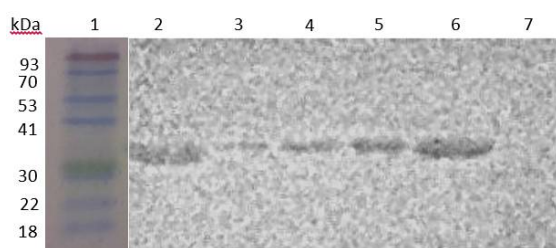
### Molecular identification of Actinobacteria

The five strains (L-asparaginase producers) were identified as *Kitasatospora atroaurantiaca* (4B-410), *Streptomyces seoulensis* (4B-411), *Streptomyces griseoluteus* (4B-412), *Streptomyces panaciradicis* (4B-413) and *Streptomyces* sp. (5C-486), using Chromas<sup>®</sup> [13] and Bioedit<sup>®</sup> to

obtain the consensus sequences, which were aligned using BLAST® [22]. Each strain presented identities greater than 99% with the sequences deposited in GenBank.

**Western blotting**

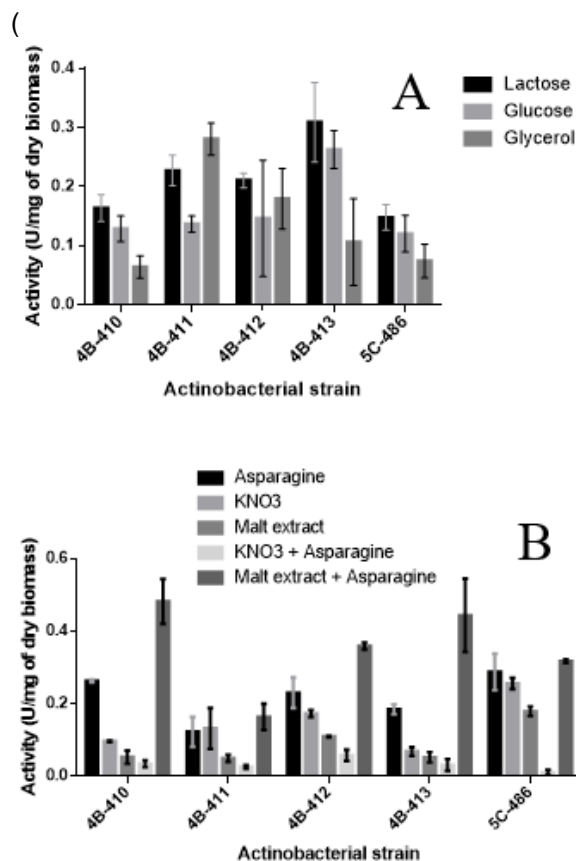
Bands with a molecular weight of 37 kDa were detected for the five strains (as an example Figure 3 show western blotting for *S. panaciradicis*). The presumed L-asparaginase bands migrated similarly to the positive control (Figure 3, lane 2); therefore, we hypothesized that the L-asparaginase enzymes from the isolated *Actinobacteria* had a similar molecular mass to the L-asparaginase from *E. coli*. In addition, the intensity of the band increased after each optimization step.



**Figure 3:** Western blotting analysis for L-asparaginase from *Streptomyces panaciradicis*. 1: Molecular weight markers (Opti-Protein XL Marker, abm®, USA); 2: Positive control, commercial L-Asparaginase® (Medac); 3: Initial broth; 4: Best carbon source broth (1 % lactose); 5: Best nitrogen source broth (0.5 % asparagine + 0.5 % malt extract); 6: Optimum conditions broth; 7: Negative control (broth with only L-glutaminase activity)

**Effect of carbon and nitrogen sources on L-asparaginase activity**

The nesslerization experiment showed that the best carbon source to maximize the L-asparaginase activity was lactose



**Figure 4A)** and the best nitrogen source was the combination of L-asparagine with malt extract. *Kitasatospora atroaurantiaca*, *Streptomyces seoulensis*, *Streptomyces griseoluteus*, *Streptomyces panaciradicis*, and *Streptomyces* sp. showed maximum L-asparaginase activities of 0.48, 0.16, 0.36, 0.44, and 0.32 U/mg, respectively

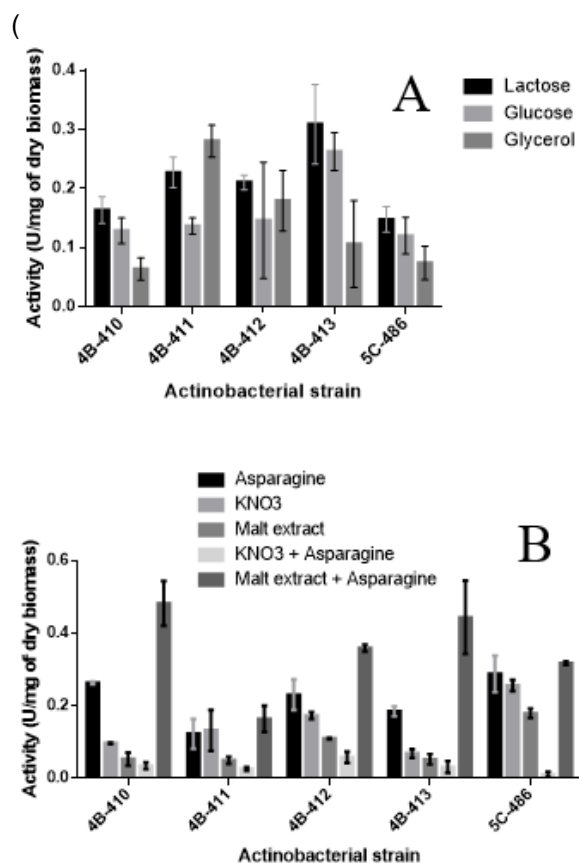
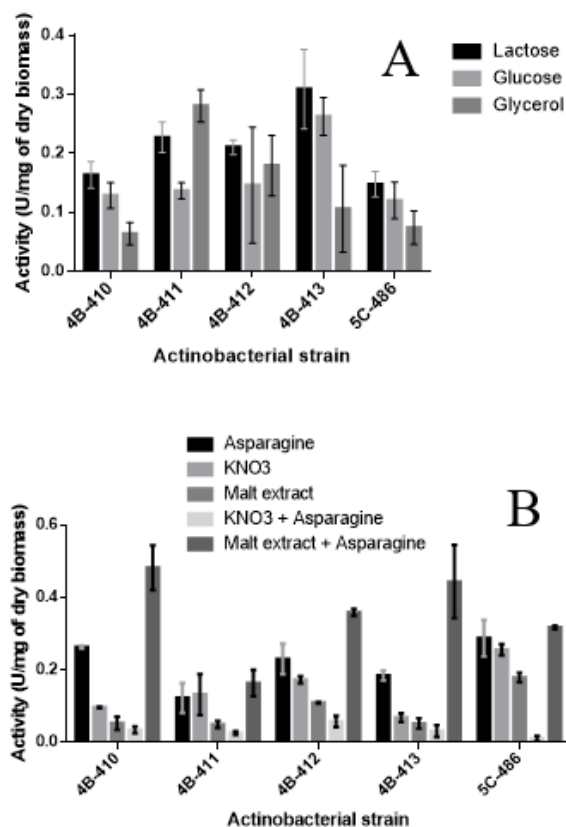


Figure 4B). *Kitasatospora atroaurantiaca*, *Streptomyces griseoluteus*, and *Streptomyces panaciradicis* were selected for further study.

### Optimized nutritional and growth factors

Experiments were randomized using the design matrix and the experimental responses of the L-asparaginase specific activity for the different trials were assessed (Table 2). The regression equations were obtained ANOVA analysis (Table 1).



**Figure 4:** Nessler assay employing different carbon sources (A) and different nitrogen sources (B) in the medium at 1 % w/v for each strain

**Table 2:** Central composite design together with the experimental L-asparaginase activity A: carbon source concentration, B: nitrogen source concentration

| Run | A %w/v | B %w/v | Temp °C | pH  | Activity (U/mg protein)  |                        |                         |
|-----|--------|--------|---------|-----|--------------------------|------------------------|-------------------------|
|     |        |        |         |     | <i>K. atroaurantiaca</i> | <i>S. griseoluteus</i> | <i>S. panaciradicis</i> |
| 1   | 5      | 1      | 30      | 7   | 6.79                     | 4.50                   | 2.16                    |
| 2   | 1      | 1      | 30      | 7   | 7.83                     | 18.34                  | 4.24                    |
| 3   | 1      | 1      | 30      | 7   | 9.65                     | 20.30                  | 1.46                    |
| 4   | 1      | 1      | 30      | 8   | 8.64                     | 3.88                   | 6.85                    |
| 5   | 0.5    | 2.5    | 32.5    | 7.5 | 11.44                    | 9.51                   | 5.68                    |
| 6   | 0.5    | 0.5    | 27.5    | 6.5 | 3.20                     | 30.72                  | 7.58                    |
| 7   | 2.5    | 0.5    | 32.5    | 7.5 | 9.51                     | 18.63                  | 28.03                   |
| 8   | 1      | 1      | 30      | 7   | 6.66                     | 3.00                   | 2.34                    |
| 9   | 2.5    | 2.5    | 27.5    | 6.5 | 6.03                     | 2.24                   | 1.52                    |
| 10  | 2.5    | 2.5    | 32.5    | 6.5 | 10.00                    | 7.54                   | 5.21                    |
| 11  | 1      | 1      | 30      | 7   | 15.05                    | 10.79                  | 2.60                    |
| 12  | 0.1    | 1      | 30      | 7   | 2.43                     | 1.62                   | 0.53                    |
| 13  | 0.5    | 2.5    | 27.5    | 7.5 | 2.69                     | 1.91                   | 0.95                    |
| 14  | 2.5    | 0.5    | 27.5    | 7.5 | 3.99                     | 11.56                  | 7.82                    |
| 15  | 1      | 1      | 25      | 7   | 11.11                    | 4.24                   | 5.70                    |
| 16  | 1      | 5      | 30      | 7   | 4.55                     | 2.28                   | 1.46                    |
| 17  | 1      | 1      | 30      | 6   | 12.94                    | 10.17                  | 4.61                    |
| 18  | 0.5    | 0.5    | 32.5    | 6.5 | 3.00                     | 1.72                   | 0.76                    |
| 19  | 1      | 0.1    | 30      | 7   | 5.00                     | 5.71                   | 6.56                    |
| 20  | 1      | 1      | 35      | 7   | 28.18                    | 22.88                  | 31.20                   |
| 21  | 1      | 1      | 30      | 7   | 3.88                     | 17.54                  | 2.09                    |

**Table 3:** Analysis of variance for L-asparaginase activity by a central composite design (CCD) for *Streptomyces panaciradicis*. (SS = Sum of Squares, DF = Degrees of freedom, MSS = mean sum of squares, F = variance ratio, p-value = probability)

| Variable | SS | DF | MSS | F | P-value |
|----------|----|----|-----|---|---------|
|----------|----|----|-----|---|---------|

**Table 1:** Coefficients of the statistical models obtained using ANOVA

| Variable                                    | Specific activity (U/mg) |                        |                         |
|---|--------------------------|------------------------|-------------------------|
|   | <i>K. atroaurantiaca</i> | <i>S. griseoluteus</i> | <i>S. panaciradicis</i> |
| Intercept ( $\beta_0$ )                     | 556.02                   | 343.59                 | 1163.08                 |
| Nitrogen source concentration ( $\beta_1$ ) | 2.12                     | 72.22                  | 6.67                    |
| Carbon source concentration ( $\beta_2$ )   | 23.46                    | 63.53                  | 125.85                  |
| Temperature ( $\beta_3$ )                   | -30.69                   | -21.57                 | -55.38                  |
| pH ( $\beta_4$ )                            | -31.58                   | -26.36                 | -123.83                 |
| $\beta_1 \times \beta_2$                    | -3.09                    | -2.22                  | -5.53                   |
| $\beta_1 \times \beta_3$                    | 0.00                     | -1.86                  | 0.00                    |
| $\beta_1 \times \beta_4$                    | 0.00                     | 0.00                   | 0.00                    |
| $\beta_2 \times \beta_3$                    | 0.00                     | -1.81                  | -1.48                   |
| $\beta_2 \times \beta_4$                    | -2.73                    | 0.00                   | -10.91                  |
| $\beta_3 \times \beta_4$                    | 1.05                     | 3.84                   | 2.81                    |
| $\beta_1^2$                                 | 0.00                     | -2.67                  | 0.00                    |
| $\beta_2^2$                                 | -0.45                    | -1.68                  | 0.00                    |
| $\beta_3^2$                                 | 0.41                     | 0.00                   | 0.66                    |
| $\beta_4^2$                                 | 0.00                     | -6.62                  | 3.69                    |
| R <sup>2</sup>                              | 0.726                    | 0.826                  | 0.9791                  |

All models showed statistical significance ( $p < 0.05$ ) and the lack of fit was not significant, showing correct correlation between the different studied variables and responses for strain *Streptomyces panaciradicis* (Table 3).



| Model                                       | 1304.042    | 10 | 130.4042    | 46,8946     | < 0.0001 |
|---|-------------|----|-------------|-------------|----------|
| Nitrogen source concentration ( $\beta_1$ ) | 68.94587352 | 1  | 68.94587352 | 24.79357702 | 0.0006   |
| Carbon source concentration ( $\beta_2$ )   | 138.954866  | 1  | 138.954866  | 49.9694615  | < 0.0001 |
| Temperature ( $\beta_3$ )                   | 0.267207332 | 1  | 0.267207332 | 0.096090241 | 0.7629   |
| pH ( $\beta_4$ )                            | 67.57586333 | 1  | 67.57586333 | 24.30090862 | 0.0006   |
| $\beta_1 \times \beta_2$                    | 105.5426859 | 1  | 105.5426859 | 37.95413095 | 0.0001   |
| $\beta_2 \times \beta_3$                    | 123.1318911 | 1  | 123.1318911 | 44.27937264 | < 0.0001 |
| $\beta_2 \times \beta_4$                    | 98.10808759 | 1  | 98.10808759 | 35.28058028 | 0.0001   |
| $\beta_3 \times \beta_4$                    | 98.57979866 | 1  | 98.57979866 | 35.45021197 | 0.0001   |
| $\beta_3^2$                                 | 450.3148981 | 1  | 450.3148981 | 161.9374234 | < 0.0001 |
| $\beta_4^2$                                 | 22.78244813 | 1  | 22.78244813 | 8.192780129 | 0.0169   |
| Residual                                    | 27.808      | 10 | 2.7808      |             |          |
| Lack of fit                                 | 23.514      | 6  | 3.9189      | 3.6502      | 0.1153   |
| Pure error                                  | 4.294       | 4  | 1.0736      |             |          |
| Total                                       | 1331.850    | 20 |             |             |          |

The CCD results identified the optimal values for carbon and nitrogen source concentrations, temperature, and pH ( ). The maximum theoretical L-asparaginase activity for *Kitasatospora atroaurantiaca* was 38.7 U/mg

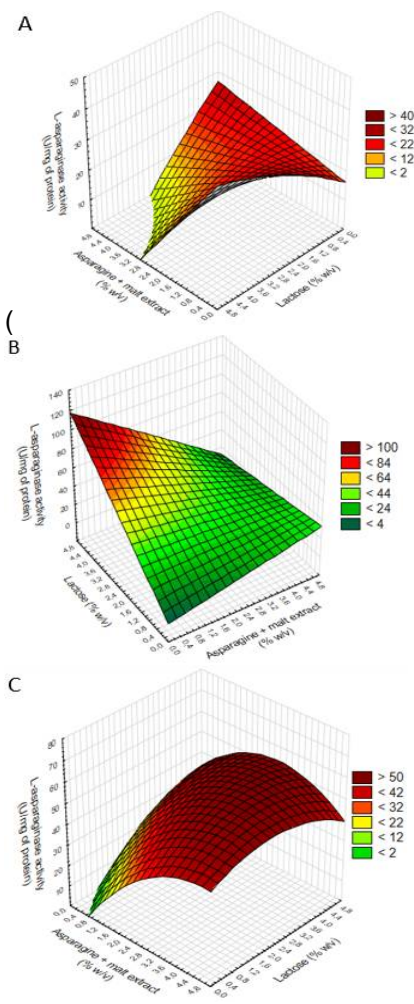
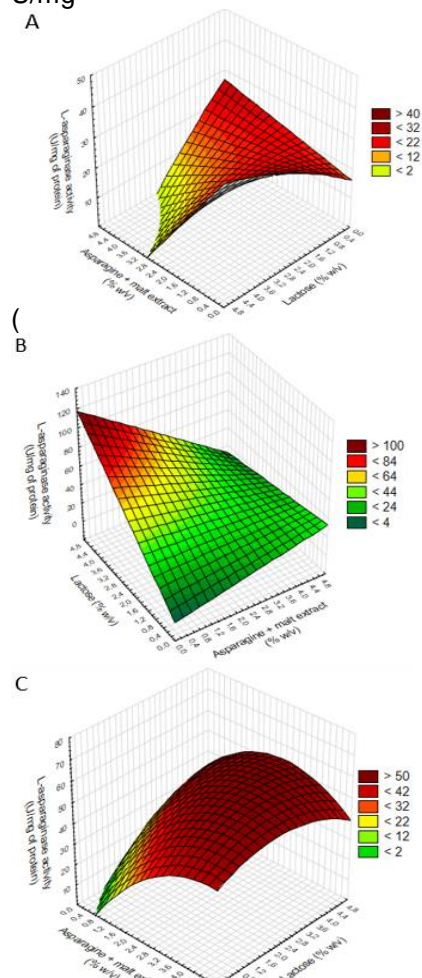


Figure 5A), for *Streptomyces griseoluteus* was 57.37 U/mg

Figure 5B), and for *Streptomyces panaciradicis* was 114.94 U/mg

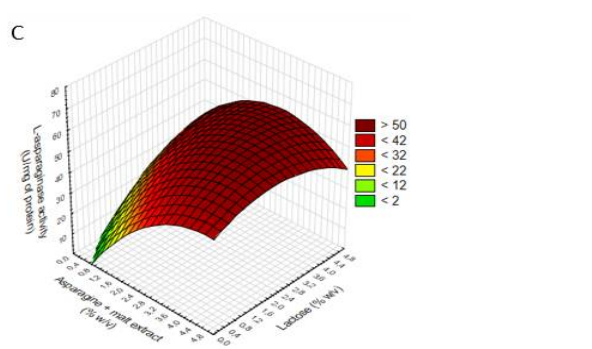
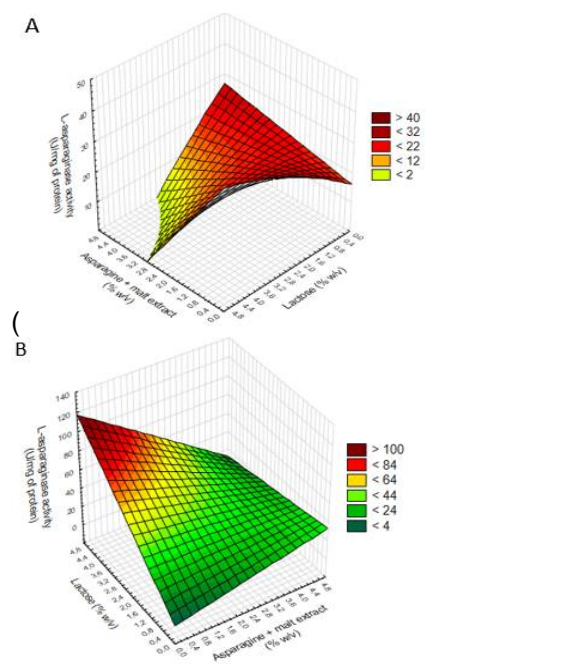


Figure 5C), indicating that *Streptomyces panaciradicis* had the highest L-asparaginase activity.

An experiment was carried out under the optimum conditions identified by the CCD to determine the accuracy of the models and to verify the results (

). The L-asparaginase activity of *Streptomyces panaciradicis* (114.06 U/mg) obtained from the experiment was very close to the predicted response (114.94 U/mg) from the regression

model, which proved the validity of the model. The verification revealed that the model was very accurate, with an error rate of only 0.77 % under the tested conditions (

**Table 4:** Verification of the accuracy of the models obtained with CCD. A: Carbon source concentration, B: Nitrogen source concentration

| Strain                   | A (% w/v) | B (% w/v) | Temp °C | pH | Activity (U/mg protein) |           | Error (%) |
|--------------------------|-----------|-----------|---------|----|-------------------------|-----------|-----------|
|                          |           |           |         |    | Experimental            | Predicted |           |
| <i>K. atroaurantiaca</i> | 0.1       | 5         | 25      | 6  | 29.4                    | 23.28     | 26        |
| <i>S. griseoluteus</i>   | 5         | 0.0       | 25      | 6  | 34.08                   | 34.66     | 1.7       |
| <i>S. panaciradicis</i>  | 5         | 0.1       | 25      | 6  | 114.06                  | 114.94    | 0.8       |

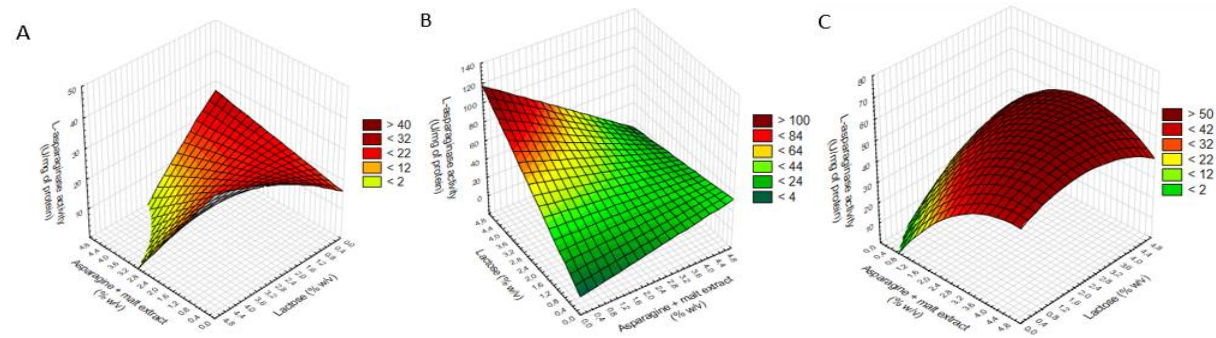


Figure 5: Surface plots for interactions between nitrogen and carbon source concentrations with L-asparaginase activity for A: *Kitasatospora atroaurantiaca*, B: *Streptomyces griseoluteus*, and C: *Streptomyces panaciradicis*

**Table 5:** Cytotoxic activity of broths with L-asparaginase activity on the MDA cell line and the IC<sub>50</sub> (ppm) value for each strain

| Concentration (ppm) | <i>K. atroaurantiaca</i> |                            | <i>S. griseoluteus</i> |                            | <i>S. panaciradicis</i> |                            | Doxorubicin Cytotoxicity (%) |
|---------------------|--------------------------|----------------------------|------------------------|----------------------------|-------------------------|----------------------------|------------------------------|
|                     | Cytotoxicity (%)         | Enzymatic activity (mU/mL) | Cytotoxicity (%)       | Enzymatic activity (mU/mL) | Cytotoxicity (%)        | Enzymatic activity (mU/mL) |                              |
| 10                  | 41.2 ± 0.76              | 0.307                      | 22.2 ± 5.51            | 0.207                      | 59.3 ± 3.40             | 0.236                      | 19.3 ± 0.35                  |
| 20                  | 45.9 ± 2.50              | 0.614                      | 34.6 ± 4.47            | 0.414                      | 64.7 ± 3.38             | 0.471                      | 62.9 ± 0.05                  |
| 50                  | 55.7 ± 4.38              | 1.535                      | 36.6 ± 5.06            | 1.035                      | 60.5 ± 5.90             | 1.178                      | 78.0 ± 0.02                  |
| 100                 | 47.8 ± 5.67              | 3.07                       | 34.7 ± 6.52            | 2.07                       | 64.0 ± 0.70             | 2.356                      | 76.3 ± 0.09                  |
| 200                 | 56.2 ± 1.18              | 6.139                      | 53.1 ± 2.20            | 4.139                      | 66.7 ± 0.56             | 4.713                      | 80.6 ± 0.02                  |



|                        |              |       |               |       |             |       |             |
|------------------------|--------------|-------|---------------|-------|-------------|-------|-------------|
| IC <sub>50</sub> (ppm) | 25.61 ± 2.15 | 0.786 | 165.29 ± 1.06 | 3.421 | 8.18 ± 1.61 | 0.190 | 1.95 ± 0.25 |
|------------------------|--------------|-------|---------------|-------|-------------|-------|-------------|

The results are expressed as mean ± SD (n = 6)

### Cytotoxic activity

MDA cells exposed to diverse concentrations of protein broths with L-asparaginase activity showed a dose-dependent decrease in viability compared with untreated cells after 48 h of treatment. The protein broths from the strains presented a half maximal inhibitory concentration (IC<sub>50</sub>) between 1 and 200 ppm on the MDA cell line (Table 5). The IC<sub>50</sub> value of doxorubicin was less than 10 ppm against the L929 cell line. The IC<sub>50</sub> values for each enzymatic broth were greater than 200 ppm against L929 cells.

### DISCUSSION

When used in chemotherapy, L-asparaginase caused problems related to its side effects associated with its L-glutaminase activity.

In the present study, we found that L-asparagine and malt extract stimulated L-asparaginase activity more than using one nitrogen source alone. However, for all the strains evaluated, L-asparagine was not essential to obtain L-asparaginase activity, as seen when malt extract or potassium nitrate alone were used as nitrogen sources.

For *Kitasatospora atroaurantiaca* and *S. panaciradicis*, temperature showed a quadratic relationship with the L-asparaginase activity, whereby there was a maximum value beyond which an increase in the factor did not correspond to an increase in activity. The concentrations of carbon and nitrogen sources showed an inverse correlation with the L-asparaginase activity in *Streptomyces panaciradicis*.

The L-asparaginase activities obtained from *Streptomyces panaciradicis* (114.06 U/mg), *Streptomyces griseoluteus* (34.08 U/mg), and *Kitasatospora atroaurantiaca* (29.4 U/mg) were much greater than those attained from other microorganisms, such as *Aspergillus terreus* (10.97 U/mg) [10], *Streptomyces thermoluteus* (4.6 U/mg), and *Streptomyces avermitilis* (5.6 U/mg) [26]. Kumari et al [17] reported the production of L-asparaginase from *Streptomyces griseoluteus* isolated from marine sediments, with an enzymatic activity of 16.88 U/mg before purification. Therefore, the three strains identified in the present study produced L-asparaginase with high activity and low levels of L-glutaminase activity.

Therefore, in the present study, we identified five *Actinobacterial* strains with high L-asparaginase and no L-glutaminase activity.

Different carbon sources have different effects on L-asparaginase production from *Streptomyces*. *S. longsporusflavus* and *S. albidoflavus* produced the maximum amount of L-asparaginase when grown on soluble starch [13,23] whereas *S. phaeochromogenes* and *S. tendae* produced the maximum activity on glycerol and sucrose, respectively [24]. However, few studies used lactose for *Actinobacterial* L-asparaginase production. Furthermore, the nitrogen sources used during fermentation has an impact on L-asparaginase production [1,2,19]. El-naggar et al [25] suggested that L-asparaginase activity increases with the concentration of L-asparagine in the medium. In

The *Streptomyces panaciradicis*, *Streptomyces griseoluteus*, and *Kitasatospora atroaurantiaca* L-asparaginases displayed similar cytotoxic activities against MDA cells (0.190, 3.421, and 0.786 mU/mL, respectively) compared with other bacterial L-asparaginases. L-asparaginase purified from *Enterobacter cloacae* showed an IC<sub>50</sub> value of approximately 11.8 U/mL [27] and Pokrovskaya et al [28] reported that the recombinant L-asparaginase produced by *Yersinia pseudotuberculosis* showed an IC<sub>50</sub> value of 10 U/mL.

### CONCLUSION

*Streptomyces griseoluteus*, *Kitasatospora atroaurantiaca*, and *Streptomyces Panaciradicis* (the last two reported for the first time as L-asparaginase producers) are potential sources of L-asparaginase with low L-glutaminase activity. Optimizing the nutritional and growth factors of *Kitasatospora atroaurantiaca*, *Streptomyces griseoluteus*, and *Streptomyces panaciradicis* increases L-asparaginase activity between 9 and 90 times. L-asparaginases from these strains possess good cytotoxicity against MDA-MB-231 cells *in vitro* and low performance on the untransformed cell line L929, suggesting they are good candidates for the treatment of acute lymphoblastic leukemia.

### DECLARATIONS

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## Conflict of Interest

No conflict of interest associated with this work.

## Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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