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Characterization and optimization of extracellular L-Asparaginase production by selected Actinomycete strain isolated from an algerian wheat bran

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Abstract: L-Asparaginase is an enzyme that hydrolyses the amino acid L-Asparagine into aspartic acid and ammonia. As a medication, L-Asparaginase is used in chemotherapy to treat acute lymphoblastic leukaemia by depleting circulating Asparagine and depriving tumor cells. Interest in Actinomycetes as potential producers of antibiotics and enzymes encouraged us to investigate an isolated strain (*CA01*) from soft wheat bran. The Actinomycete strain was characterized based on its morphological and biochemical characteristics and selected due to a proved promising ability to produce L-Asparaginase optimized in both solid and liquid media cultures. The conditions of enzyme production were standardized according to a one-factor-at-a-time (OFAT) experimental design. To obtain optimal medium combination, a Box-Behnken Response Surface Methodology (RSM) has been adopted by choosing the most influential factors. The optimal conditions for the enzyme production were (g/l): L-Asparagine 10.7; Glucose 2.7; starch 7, in based medium containing (g/l): K_2HPO_4 0.5; $MgSO_4 \cdot 7H_2O$ 0.1, corresponding to an optimal enzymatic activity of 8.03 IU/ml at 27.83°C. The maximum production of enzyme was reached on the sixth day of experiment. The ANOVA test (P value < 0.05) and adjusted R^2 values close to the experimental R^2 show that the obtained model of the active L-Asparaginase of *CA01* strain production is significant with the following linear terms: temperature, substrate concentration, Glucose concentration and there squared.

Key words: L-Asparaginase; Actinomycetes; Enzyme; Optimization; Box Behnken Design; RSM.

Introduction

L-Asparaginase (L-Asparagine amidohydrolase EC.3.5.1.1) is the enzyme that catalyses the hydrolysis of amid group in the side chain of L-Asparagine to yield L-aspartate and ammonia (1). L-Asparaginase is nowadays recognized as a therapeutic enzyme used in anticancer chemotherapy, in particular in the treatment of acute lymphoblastic leukaemia. The transformed lymphocytes unlike normal cells are unable to produce their own L-Asparagine because of their Asparagine synthetase deficiency. For this, these cells use L-Asparagine from the plasma and surrounding tissues to ensure their growth (2). As a result, administration of L-Asparaginase and the hydrolysis of L-Asparagine from the medium deprives the transformed lymphocytes of L-Asparagine which is considered as a growth factor and therefore essential for their development (3), and as a result, most of the cells die (4). This molecule is also used in food industry sector to prevent the formation of acrylamide recognized as a carcinogenic compound formed in foods at high temperatures (5). L-Asparaginase is marketed under the names: ELSAPAR, ERWINASE, KIDROLASE and ONCASPAR. For clinical use, only the enzyme produced by microorganisms that is exploited. Today, it is the enzyme produced by *E. coli*

that dominates the market, sold in Germany under the name of GRASNTIN and in Japan under the name of LEUMASE (6).

There are several types of L-Asparaginase which differ in particular according to their location in bacterial cells. We can find intracellular enzymes which are most often located in the periplasmic space, between the plasma membrane and the bacterial wall (7) as well as an extracellular enzymes secreted in the growth medium. This last type of L-Asparaginase is the subject of the present study in which the conditions of its production by a bacterial strain belonging to Actinomycetes is standardized and optimized. Actinomycetes are known for their biotechnological uses, especially in the pharmaceutical field where 70% of conventional antibiotics are produced from them (8). They are also powerful producers of enzymes for industrial and therapeutic interests (9). Our strain is isolated from the soft wheat bran. We chose this product in order to promote it because it is not valued locally. At the beginning, we looked for the cellulase activity, an enzyme responsible for the hydrolysis of cellulosic polymers on the walls of vegetable seeds (10). More specifically, the present paper focused on the research and the optimization of the L-Asparaginase production by the Actinomycete strain

CA01 with interesting activities.

Materials and Methods

Isolation of the bacterial strain

The selected Actinomycete *CA01* strain isolated from a sample of soft wheat bran obtained from a local Algerian Mill (Tizi-Ouzou region) was air-dried at room temperature for 21 days to target Actinomycetes spores and eliminate unwanted microorganisms (11). The wheat bran was then crushed and sieved to achieve the finest powders. A stock solution preparation was performed according to (12) where 1g of fine powder was dispersed in 9 ml of sterile physiological water. Then, a series of decimal dilutions is carried out up to a dilution of 10^{-3} (13). The isolation was performed by inoculating 1 ml of the 10^{-3} dilution on an ISP2 (International Streptomyces Project 2) (14), an agar supplemented with 100 µg/ml nalidixic acid to inhibit Gram-negative bacteria and nystatin with 50 µg/ml to prevent the development of fungi. (14). the culture was incubated for 7 days at 28°C (15, 16). Typical Actinomycete colonies have been partially identified based on their morphological, biochemical and physiological characters.

Detection of L-Asparaginase activity

On agar medium

The production of L-Asparaginase was revealed using medium Asparagine Dextrose Salt agar (ADS) (17) which contains L-Asparagine as the unique source of Nitrogen and the following chemicals (g/L): L-Asparagine 10; D-Glucose 2; K_2HPO_4 0,5 ; $MgSO_4 \cdot 7H_2O$ 0,1 ; phenol red 0,09 (18). The culture was incubated at 28°C for 7 days. The change of coloration from yellow to pink around bacterial colonies indicates the alkalisation of the medium due to the presence of ammonia produced by L-Asparagine hydrolysis reaction catalyzed by L-Asparaginase. The pink color corresponds to the turn of the pH indicator. Alkalisation is shown by a color change of the Phenol Red (18).

On liquid medium

Following the same method, the ADS broth was inoculated with strain *CA01* and incubated at 28°C for 7 days. The change of color medium from yellow to pink indicates the production of ammonia following the hydrolysis of L-Asparagine.

Quantification of L-Asparaginase activity

The applied approach is a modification of the Nesslerization protocol. The parameters have been standardized after several tests based on significant related studies. To allow an oxygen supply in aerobic culture, a volume of 100 ml of ADS broth was prepared in 250ml. An inoculum size of 10^7 UFC/ml of the isolated strain was added to the culture and incubated at 28°C for 7-10 days. After centrifugation of the bacterial culture at 10000 g for 20 min at +4°C, the obtained supernatant was used as a crude extract for the enzymatic activity assay. This test is based on the detection of ammonia produced by L-Asparagine hydrolysis using Nessler's reagent. 0.2 ml of the crude extract were mixed with 0.9 ml of the substrate (L-Asn: 0.04 M solubilized in

Tris/HCl: 0.5 M, pH 7.2) and incubated at 37°C for 20-30 min. The reaction was stopped by adding 1.5 M trichloroacetic acid. Then; 0.2 ml of the mixture was diluted with 1 ml of distilled water and mixed with 0.5 ml of Nessler's reagent. The resulting reacting mixture was incubated at room temperature for 10-15 minutes before reading the absorbance by spectrophotometry at 450 nm. The blank was treated the same way except that the enzymatic extract was replaced by the ADS broth (Asparagine Dextrose Agar) (19, 20, 11). A calibration curve was plotted using a series of ammonium sulphate solution with known concentration as source of ammonia (15).

Standardization of the medium for L-Asparaginase production

We adopted an OFT experience plan to study the effects of adding each of the following substrates in the ADS medium : Carbon sources (0.2% Glucose; 2% starch; 0.1% Glucose + 0.7% starch) and Nitrogen sources (organic : 1% L-Asparagine or mineral : 1% $NaNO_3$).

Kinetic study of L-Asparaginase production

The kinetic of L-Asparaginase production by the Actinomycete strain *CA01* was carried out. This approach was also used to standardize the incubation time. The production of the active enzyme was estimated each time by the method of Nessler.

Optimization of the medium for L-Asparaginase production

After defining the types of Carbon and Nitrogen sources and the incubation time in the standardization step, we adopted an DOE (Design of experiments approach) to evaluate the main effects and interaction of the independent variables (factors) which are: temperature ($T^{\circ}C$), substrate concentration of L-Asparagine (SC%) and Glucose concentration (GC%) on the production of L-Asparaginase by the strain *CA01*. It is useful to consider the factor response relationship in terms of a mathematical model such as the response function. The use of variance analysis and response surface design of experiments allowed us to express the L-Asparaginase activity as a polynomial model. Three factors to be investigated at limit points (-1; +1): T (25°C, 30°C); SC (0.75%, 1.25%); GC (0.1%, 0.3%) given 12 combinations and 3 points in the domain center (0; 0) were performed. A plan of 15 experiments was studied and the corresponding design matrix is shown in the table 2. The response value in the matrix is the average value obtained in three parallel experiments. The order in which the experiments were made was randomized to avoid systematic errors and the results were analyzed with Minitab17® software. The model which can describe the behavior of the system can be expressed by a quadratic equation 1 :

$$Y = a_0 + \sum a_i x_i + \sum a_{ii} x_i^2 + \sum a_{ij} x_i x_j \quad (1)$$

Where x is the factor in the coded value, Y is the predicted response in actual value, a_0 the intercept term, a_i the linear effect, a_{ii} the squared effect, and a_{ij} is the interaction effect. The regression equation was optimized

for maximum value to obtain the optimum conditions giving the maximum yield of L-Asparaginase.

Results

Isolated Actinomycete strain

Specific colonies of Actinomycetes were successfully isolated using appropriate selective media (ISP2). For an effective development, the colonies were maintained on this rich malt extract as reported in several studies (21). We have isolated our strain by a serially diluted samples, after obtaining an initial solubilisation of 1g of soft wheat bran in 9 ml of physiological water. It should be noted that literature data concerning the isolation of Actinomycetes strains from wheat bran are not available which implies that there is no work already done in this direction. For this, our approach is based on the isolation of a very active strain from our wheat bran powder. After isolating, typical Actinomycete colonies were morphologically, biochemically and physiologically identified. The results were summarized in Table 1.

We have identified strain *CA01* as an Actinomycete after a series of observations on the colonies : powdered appearance on the surface of agar plate (Fig.1-A) , solidity and binding on the ISP2 medium, filamentous hyphae structure (Fig.1-B) , spore production, presence of aerial mycelium and substrate , and finally Gram + character (Table 1).The studied characters are presented in Bergey’s manual of systematic bacteriology (2012), in section Actinobacteria.

By comparing the results obtained with the negative control, the change from the yellow colour of the ADS medium to the pink, around the bacterial colonies, indicates the alkalisation of the medium confirming the production of ammonia by L-asparagine hydrolysis owing to L-Asparaginase production (19). The same test in the liquid medium showed the same results (Fig.3). In similar work aimed at researching and optimizing

the production of L-Asparaginase by Actinomycetes, the ADS medium supplemented with phenol red as a pH indicator is the most used for the screening of enzy-

Table 1. Biochemical and physiological characteristics of the identification of the selected Actinomycete strain *CA01*.

Studied Characteristics	<i>CA01</i> Strain
Sugars and derived degradation	
Glucose	+
Fructose	-
Lactose	+
Starch	+
Citrate	+
Mannitol	+
Gas production	-
H ₂ S production	-
Indole production	-
Sugars attack pathway (MEVAG)	
Aerobic	+
Anaerobic	-
Urease	-
Catalase	+
Oxidase	-
Tryptophane Deaminase (TDA)	+
Nitrate Reductase	+
VP	+
RM	-
Caseins hydrolysis	+
Mobility test	-
Growth at different temperatures	
4°C	-
25°C	+
28°C	+
37°C	+
44°C	-
50°C	-
Growth at different pH	
5	-
6	+
7	+
8	+
9	+
10	-
11	-
Tolerance of different concentrations of NaCl	
5%	+
10%	+
15%	+
20%	+
25%	-
30%	-
Morphology and color of spores	Round colonies, spores located on the Aerial Mycelium (AM)
Color of Substate Mycelium (SM) / Color of Aerial Mycelium.	Greyish/White
Appearance of spore mass	Powdery, greyish
Gram	Gram +

+ : Presence of the studied character; - : Absence of the studied character.

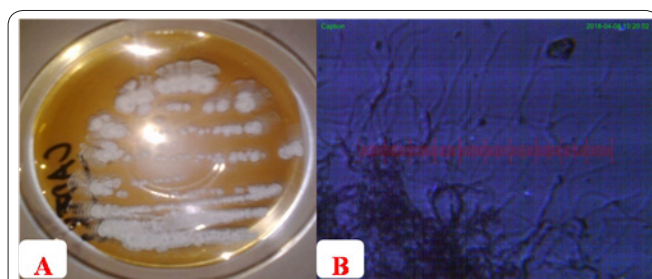


Figure 1. Morphological characteristics of strain *CA01*. **A:** Macromorphology and colony appearance. **B:** Micromorphology and filamentous hyphae observed under light microscopy CETI with G×100.

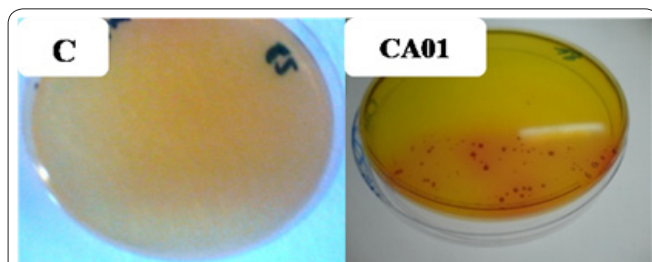


Figure 2. Detection of L-Asparaginase production in ADS agar medium. **C:** negative control represented by ADS non-inoculated agar; **CA01:** *CA01* strain in ADS agar medium.



Figure 3. Detection of L-Asparaginase production in ADS broth medium. C: negative control containing the ADS broth non-inoculated S: CA01 strain in ADS broth medium.

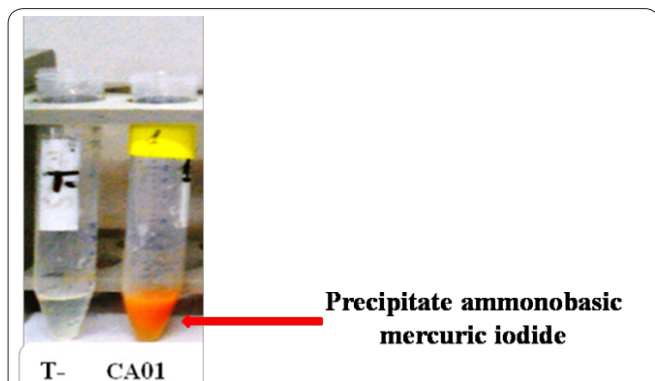


Figure 4. Results of quantification of L-Asparaginase activity according to the Nessler assay protocol. T- : Blank of the enzymatic reaction: no inoculated ADS broth + substrate (L-Asparagine 0,04M); CA01: culture supernatant of CA01 + substrate (L-Asparagine 0,04M).

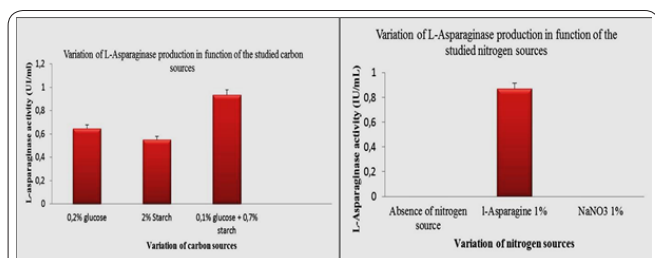


Figure 5. Variation in L-Asparaginase production expressed in terms of enzyme activity (IU/ml) as a function of the source type of Carbon (A) and Nitrogen (B).

matic activity following the turn from the middle color. In the work of Konečná and *al.*, (21), an Actinomycete strain of the genus *Arthrobacter* was isolated in ISP2 medium and had screened the L-Asparaginase activity on modified ADS agar. The same authors optimized the production of the enzyme on ADS broth taking into account the temperature and the chemical composition of the medium.

The L-Asparaginase activity assay was performed by the Nessler reagent (19, 22). The appearance of an orange-yellow precipitate (Fig.4) in the tube containing the crude extract of the bacterial culture, unlike the control tube (T-), is due to the formation of a chemical complex ammonobasic mercuric iodide between the Nessler reagent (composed of potassium iodide KI

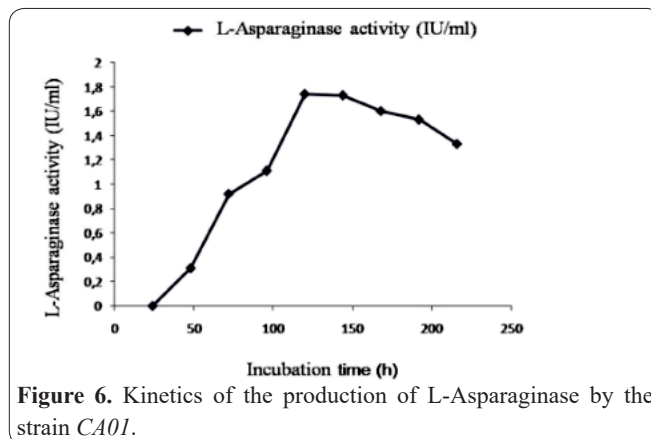
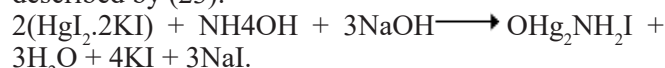


Figure 6. Kinetics of the production of L-Asparaginase by the strain CA01.

and mercuric iodide HgI₂, in a basic medium containing NaOH) and ammonia NH₃. The reaction was yet described by (23):



Ammonia comes from the hydrolysis reaction of L-Asparagine by L-Asparaginase action. For this, we can support our initial hypothesis on the production of the targeted extracellular L-Asparaginase enzyme by our strain CA01.

Standardization of the medium for L-Asparaginase production

Effect of Carbon and Nitrogen sources on L-Asparaginase production

By changing the Carbon source, it was found that the production of L-Asparaginase is higher (0.93IU/ml) using the combination of 0.1% Glucose / 0.7% starch. In contrast for the same strain, the production is decreasing significantly (0.64IU/ml) by using 0.2% Glucose and usual ADS medium (Fig. 5-A).

By using a starch alone at 2%, we observed for strain CA01 an enzymatic activity of 0.55 IU/ml. This was probably due to the high capability of the bacterial strain on degrading the carbohydrate polymers of the starch-rich wheat bran. We have standardized the enzyme production following the approach undertaken by other works (24) where better L-Asparaginase activity in a strain of *Streptomyces* were observed using the Glucose/starch combination at 2/20 ratio. However, in our study the ratio was 1/7 revealed a relatively less demanding substrate additions while maintaining a reasonable enzyme production level. This property is very interesting for developing large-scale cost-effective production methods. For the variation of the source of nitrogen, the strain produced the enzyme in the presence of L-Asparagine at 1%, the substrate of the enzyme, have an enzymatic activity of 0.87IU/ml. However, in the presence of a mineral Nitrogen source: NaNO₃ 1%, or in the absence of any source of Nitrogen, there was no L-Asparaginase production (Fig. 5-B). This finding reinforces the probably that L-Asparaginase is an inducible enzyme, since its production has been repressed in the absence of its specific substrate (Fig.5-B).

Kinetics of L-Asparaginase production and standardization of the incubation time

The graph in figure 6 represents the kinetics of L-Asparaginase production expressed as total enzyme activ-

Table 2. Box Behnken Design matrix of experiments.

StdOrder	RunOrder	Coded experiments matrix			L-ASP activ (IU/mL)
		T	SC	GC	
3	1	-1	1	0	3,11
8	2	1	0	1	2,08
11	3	0	-1	1	4,11
7	4	-1	0	1	3,61
4	5	1	1	0	1,33
10	6	0	1	-1	3,01
1	7	-1	-1	0	2,02
2	8	1	-1	0	0,48
9	9	0	-1	-1	2,14
5	10	-1	0	-1	1,64
15	11	0	0	0	7,5
6	12	1	0	-1	1
14	13	0	0	0	6,98
12	14	0	1	1	8,01
13	15	0	0	0	6,88

Table 3. Statistical parameters estimation.

Term	Effect	Coefficient	SE Coefficient	T-value	P-value	VIF
Constant		2.6679	0.0842	31.63	0.000	
T	-0.5193	-0.2596	0.0517	-5.03	0.004	1.00
SC	0.4694	0.2347	0.0517	4.54	0.006	1.00
GC	0.6803	0.3402	0.0517	6.59	0.001	1.00
T²	-2.0183	-1.0091	0.0760	-13.27	0.000	1.01
SC²	-0.8020	-0.4010	0.0760	-5.27	0.003	1.01
GC²	-0.5060	-0.2530	0.0760	-3.33	0.021	1.01
T×SC	0.0591	0.0295	0.0730	0.40	0.703	1.00
T×GC	-0.0886	-0.0443	0.0730	-0.61	0.571	1.00
SC×GC	0.2654	0.1327	0.0730	1.82	0.129	1.00

ity (IU/ml). On a usual ADS medium, the production of the enzyme started from day 02 (48h) and increased exponentially until day 06 (144h) with a value of 1.73 IU/ml. After 144 h the production decreased significantly.

Optimization of L-Asparaginase production

This randomized matrix represents the responses obtained during all the 15 carried out experiments where each was repeated three times. The mean value of each experiment refers to the responses.

The analysis of variance is used to determinate the statistical significant effect of the factors. According to the ANOVA (Table 4) we can note that the linear and the square effects of all the factors studied are significant with a probability *P-value* < 5%. On the other hand, the interactions of factors are not significant. From the results (Table 3), we can see that in L-Asparaginase production, the effect of temperature has a negative sign meaning that the yield of enzyme production decrease as the temperature increase in the domain of variation of the studied factors. This temperature range was chosen according to the results of (11) indicating that the production of L-Asparaginase does not necessarily require high temperature. The significance of the obtained model is evaluated with an adjusted correlation factor *R² adj* of 95.28% which is close to the real *R²* of 98.31%. This demonstrates a perfect correlation of the experimental

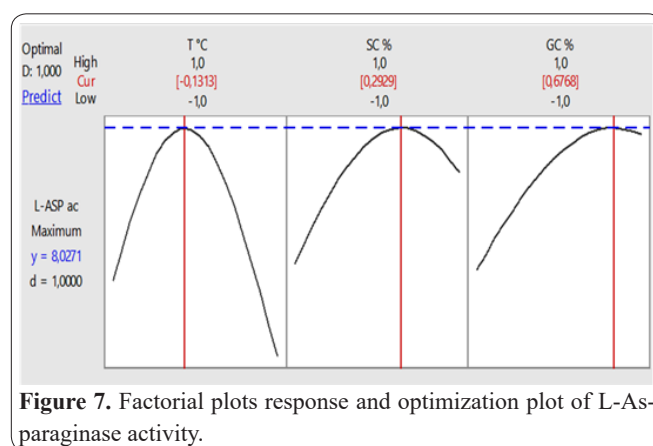


Figure 7. Factorial plots response and optimization plot of L-Asparaginase activity.

values with the values adjusted by the model. However, the non-significant variables should be eliminated in order to obtain a representative model of the response studied, with a better correlation between L-Asparaginase activity and the factors. The reduced model can be expressed in according to the equation 2 as:

$$Y = 2.6679 - 0.2596T + 0.2347SC + 0.3402GC - 1.0091T^2 - 0.4010SC^2 - 0.2530GC^2 \quad (2)$$

Optimization

The figure 7 illustrates the maximum response obtained by the model as a function of the optimal values of the chosen variables. The optimal enzymatic activity

Table 4. Analysis of Variance for L-Asparaginase.

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	9	6.22248	0.69139	32.39	0.001
Linear	1				
T	1	0.53930	0.53930	25.27	0.004
SC	1	0.44069	0.44069	20.65	0.006
GC	1	0.92568	0.92568	43.37	0.001
Square	1				
T²	1	3.76002	3.76002	176.17	0.000
SC²	1	0.59375	0.59375	27.82	0.003
GC²	1	0.23637	0.23637	11.07	0.021
2-Way interaction	1				
T×SC	1	0.00349	0.00349	0.16	0.703
T×GC	1	0.00785	0.00785	0.37	0.571
SC×GC	1	0.10672	0.10672	3.30	0.129
Error	5	0.10672	0.02134		
Lack-of-fit	3	0.09903	0.03301	8.58	0.106
Pure Error	2	0.00769	0.00385		
Total	14	6.32920			

S = 0.146094 ; R² = 98.31% ; R² adjusted = 95.28% ; R² predicted = 74.69%

obtained through our approach is then 8.0271 IU/mL at the optimal coded values of the factors: T = -0.1313, SC = 0.2929, GC = 0.6768. These values correspond to the actual optimum values: Temperature: 27.83°C; L-Asparagine: 1.07%; Glucose: 0.27%.

Discussion

In order to demonstrate the production of L-Asparaginase, strain CA01 was cultured on ADS medium. The same medium is used in many studies related to the production and characterization of L-Asparaginase (25, 26, 27, 28, 29, 30, 31). The production yield of the enzyme depends substantially on the physicochemical conditions of the bacterial culture which implies an optimization of all the factors that can influence this production. In our study, we started by normalizing Carbon and Nitrogen sources and incubation time. The study was performed by adopting a surface methodology using Box–Behnken experimental design (BBD) to optimize temperature and concentration of the substrate and Glucose. The using of BBD showed that the pH, starch, yeast extract and L-asparagine concentration were the factors directly correlated with the production of L-Asparaginase by strain *Streptomyces griseus* NIOT-VKMA29 (11). The use of Glucose at 2.0% as the only source of Carbon exhibited a low L-Asparaginase production comparatively to 1.0%. This can be explained by the acidity of fermentation medium hindering enzyme production (32, 33). Glucose is known to lower the enzyme yield by acting as a repressor (34, 35). Thus, the addition of starch in the culture would be positive for increasing this productivity due to the alkalization of the medium (11). In the same context, it has been reported that starch is the best source of Carbon for the production of L-Asparaginase by the strain *Streptomyces longporus flavus* (27). Muvva *et al.* (36) concluded that starch with Glucose is the best source of Carbon for maximum L-Asparaginase production. This supports our results where we

obtained a better yield of the enzyme in the presence of the combination 0.1% Glucose / 0.7% Starch. Interestingly, Agarwal *et al.* (37) insisted that the bacteria use Glucose preferentially during the first fermentation period and then the acidity generated by its degradation is often neutralized by the production of ammonia from hydrolysis of L-Asparagine. After conducting the BBD study in a basic medium containing starch (0.7%) and Glucose at a variable rate (0.1 to 0.3%), it was found that the optimal concentration of Glucose for optimal L-Asparaginase was 0.27%. The addition of starch at 0.7% revealed a positive effect (+0.68) of Glucose on the enzyme production (see Table 1 for additional statistical parameters estimation). Meena *et al.* (11) obtained close results to our values (8.03 IU/mL) with an optimal production of 8.93 IU/mL in the presence of 1% starch in the ADS medium. Regarding the Nitrogen source, we recorded an absence of L-Asparaginase production in the absence of its substrate (L-Asparagine). The positive effect (+0.47) of the substrate L-asparagine is interesting because after optimization, we have deduced an optimal value of 1.07% allowing to have a maximum of production of the enzyme (8.03 IU/mL). Similar values were found in the production of L-Asparaginase by *S. griseus* NIOT-VKMA29 at 1.5% of asparagine (11). Kiranmayi *et al.* (2013) (38) concluded that L-Asparagine is the best Nitrogenous substrate that has resulted in optimal production of L-Asparaginase on ADS broth by an actinomycete strain, *Pseudonocardia endophytica* VUK-10. L-Asparagine is also considered as an inducible substrate in the ADS culture medium, which produced an optimal of L-Asparaginase, by an Actinomycete strain, *Streptomyces ginsengisoli* at a concentration of 0.05% (36). In addition, the medium with high concentration of L-Asparagine prompts the actinomycetes to produce a greater amount of L-Asparaginase than under normal biological conditions (25). The production of L-Asparaginase by our strain is negatively influenced by temperature with an effect of -0.52 (Table 3).

For this, a decrease in temperature during fermentation can increase the L-Asparaginase yield. This result is expected because it is common in Actinomycetes where practically the maximal activity of L-Asparaginase activity of L-asparaginase is recorded at growth temperature (28°C). of incubation on ADS medium. In our study, Production of L-Asparaginase reached the maximum after 144 h of incubation (6th day). We assume that the maximum enzyme production growth was achieved during the stationary growth phase. Ramesh and Lonsane (40) obtained a maximum of L-Asparaginase at 51 hours of incubation of *Serratia marcescens* SB08 that corresponds to the stationary phase of the growth. According to the same authors, the decrease of the enzymatic level from this phase is due to its interaction with the molecules resulting from the cells lysis. Furthermore, it has been proved that the maximum production of L-Asparaginase on the ADS broth by *Streptomyces griseus* NIOT-VKMA29, was reached after 144 h (06 days) of incubation, (11, 25, 41). However, in the work of Alapati and Muvva in 2010 (42), a maximum of L-Asparaginase activity was observed after 72 hours of incubation. Of the various conditions tested in our study, temperature of 27.83 °C, an incubation time of 6 days, a rate of L-asparagine 1.07%, Glucose 0.27% and 0.7% of starch were found to be optimum for L-Asparaginase production with a significant yield of 8.03 IU/mL. The present study revealed that the experimental approach based on BBD significantly increased the enzyme production yield compared to the usual starting conditions where the maximum activity of L-Asparaginase recorded was 0.87 IU / mL.

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Author's contribution

A.C. made and performed the experiments and interpreted the results. M. K. co-directed the work and identified the bacterial strains. K.H. directed the work in his research laboratory. N.A. has done the translation and revision of the English language. A. T. realized the DOE approach. Y.T. and S.B. participated in the realization of cultures. L. B and N.K. contributed in the redaction style.

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