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OPTIMIZATION OF THE ENVIRONMENTALFACTORS AFFECTING *STENOTROPHOMONAS*SPFZL-ASPARAGINASE PRODUCTION

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ABSTRACT: *Statistically-based experimental designs were applied to optimize a solid-state fermentation for the production of l-asparaginase by a novel bacterial strain of Stenotrophomonas spFZ . Eleven culture conditions were examined for their significance on enzyme production andusing Plackett–Burman factorial design. Casein hydrolysate and magnesium sulphate were the most significant factors improving enzyme production. Maximal enzyme activity (21.23 IU) has been detected under the following conditions: Glucose, 20; Asparagine, 0; Casein 30; KCl; 0.04;NH 4SO4, 10; NaNO3,10; ZnSO4,0.36;FeSO4, 0,36; Urea,30; Maltose, 10 and MgSO4, 0.18g/l.which is more than tree folds the activity in basal medium. A verification experiment was carried out to examine model validation and revealed more than 99% validity.*

KEYWORDS:L-asparaginaseproductioN,Statistically-basedexperiments,Solid-state fermentation

INTRODUCTION

Asparaginase is an enzyme which converts L-asparagine to L-aspartic acid and ammonia (Hill et al., 1967) .It had receive a great attention in recent years because of its tumor inhibitor property especially in treating acute lymphoblastic leukermia (Verma et al., 2007) .It has also been used for treatment of lymphosacroma and in many other clinical experiment relating to tumor therapy in combination with with chemotherapy (Aguayo et al.,1999).L-asparaginase production using microbial systems has attacked considerable attention, owing to the cost-effective and eco-friendly nature. A wide range of microorganisms such as filamontous fungi, yeasts and bacteria haveproveed to be beneficial sources of this enzyme (Sarquis, et al.,2004).L-asparaginase produced throughout the world using submerged fermentation (SF) technique. Although submerged fermentation is a cost- intensive method, it is still a commonly used technique for L-asparaginase production throughout the world because of its apparent advantages in consistent enzyme production with defined medium and process condition, and advantage in downstream processing. However, the major shortcomings of L-asparaginase production by submerged fermentation are low concentration products, and consequent handling, reduction and disposal of

large volumes of water during the downstream processing (Datar,1986).Solid state fermentation has gained fresh and plentiful attention of researchers to overcome the drawbacks of submerged fermentation such as lesser energy requirements, very low risk of bacterial contamination, lower need of water and less environmental concerns regarding the disposal of solid waste (Doelle et al.,1992). Additionally, the utilization of agro-waste solid as a substrate for carbon and energy requirements under solid state fermentation makes this approach environmental friendly.

The aim of the present study is to iinvestigate the optimum environmental conditions that lead to maximum L-asparaginase production by local bacterial isolate under solid state fermentation using wheat bran in large scale industrial production to be used in the treatment of lymphoblastic leukemia.

MATERIALS AND METHODS

Chemicals

L-asparagine , Nessler reagent Sephadex C25 and Sephadex G-75-50 were obtained from sigma, Aldrich USA. Other used chemicals are of analytical grade were obtained from recognized chemical suppliers.

Isolation of Microorganisms

L-asparaginase producers were isolated from soil samples collected from different locations in Bekka Valley at 30 cm depth from soil surface under aseptic conditions.Serial dulitions of the soil sample (1/10, $1/10^2$, $1/10^3$, $1/10^4$ and $1/10^5$) were prepared in sterile distilled water and plated onModified ISP-5 Medium cotained (g/l):(L-asparagine 1, Dipotassium phosphate 1, trace salts solution 1 ml, lactose 20, phenol red 1 ml, agar 20, pH 7.0) was sterilized (autoclavedat 120°C for 20 min), inoculated with 1 ml of collected soil samples and poured to thePetri plates. All the plates were incubated at room temperature including the controlplates, which were without carbon source (lactose), and then incubated at 30◦C ± 2 for 2 days.The isolated organisms were maintained on Modified ISP-5 Medium.

Preparation of seed culture

Seed cultures were prepared in 250 ml Erlenmeyer flasks containing 50 ml nutrient broth medium with 3 ml of bacterial suspension (prepared from 18 hrs old cultures) and then incubated at 30˚ C ± 2 on a rotary shaker (180 rpm) till reaches $O.D_{600} \leq 1$.these were used as standard inocula (3ml/flask) unless otherwise stated.

Screening experiments for bacterial production of L-asparaginase

Determination of L-asparaginase

Qualitative Method (using rapid plate assay technique)

Plate assay method for screening of L-asparaginase producing microorganisms was performed according to the method adopted by Gulati et al. (1997). The modified ISP-5medium was supplemented with 1 ml of 2.5% phenol red as an indicator. The plates were inoculated with the selected isolates and incubated at 30˚ C for 48 hrs. the isolate that showed pink zone around the colonies indicated L-asparaginase production and was selected for determination of enzyme activity.

Quantitative method

Under submerged fermentation (SF)(Roberts et al.,1968)

Cultivation was achieved in 250 ml Erlenmeyer flasks each containing 5 g waste materials and 50 ml of 0.1M Phosphate buffer. The fermentation media was sterilized by autoclaving for 20 min. and inoculated with 3 ml as standard inocula. Unless otherwise stated and incubated at 30˚ C ± 2 for 24 hrs under shaken and static conditions conditions using incubator shaker (180 rpm).

Under solid state fermentation (SSF)(Ramesh & lonsane,1987)

The modified fermentation medium was dispensed in 250ml Erlenmeyer flasks each containing 5 g waste materials with 5 ml 0.1 M phosphate buffer (pH7.4). The fermentation medium were sterilized by autoclaving for 20 min. an inoculated with 3 ml as standard inocula unless otherwise stated and incubated at 30˚ C ± 2 for 24 hrs under static conditions.

Preparation of cell-free extract

Under submerged fermentation media

At the end of the fermentation period, the SF media was subjected to cooling centrifugation for 20 min at 8000 rpm. The obtained cell free extract (filtrate) was used as the crude enzyme.

Under solid state fermentation media

At the end of the fermentation period, 90 ml of 0.01 M phosphate buffer pH 7 was added to the SSF medium. The mixture was washed for 15 min using reciprocal shaker (150 rotation per minute), then subjected to cooling centrifuge for 20 min at 8000 rpm. The obtained cell free extract (filtrate) was used as crude enzyme.

Enzyme assay

L-asparaginase catalyzes the hydrolysis of L-aspargine to L-aspartate and ammonia. L-asparaginase activity was measured by direct nesslarization methods as explained by Wriston (1970). This method is based on the determination of ammonia liberated from L-asparagine by L-asparaginase enzyme in the nessler reaction. The reaction was started by adding 0.2 ml of

supernatant into 1.8 ml 0.01 M L-asparagine prepared in 0.05M Tris HCl buffer (pH 8.6) and incubated at 30˚ C ± 2 for 30 min. The reaction was stopped by the addition of 0.5 ml of 1.5 M trichloroacetic acid (TCA). The ammonia released in the supernatant was determined calorimetrically by adding 0.2 ml Nessler's reagent into tubes containing 0.1 ml supernatant and 3.75 ml distilled water and incubated at room temperature for 10 min and absorbance of the supernatant was read using UV –visible spectrophotometer at wavelength of 450 nm. The A_{480} values were measured against the blank which was not inoculated with a bacterial suspension.
One L-asparaginase unit of activity was defined as the amount of enzyme that liberates 1 µmol ammonia/ min at 30˚ C ± 2. The ammonia concentration produced in the reaction was determined on the bases of a standard curve.

Environmental factors affecting L-asparaginase production by *Stenotrophomonas*sp FZ under solid state fermentation

Effect of incubation period

The following study was carried out to follow up the enzyme production at time intervals to determine the enzyme production at which maximum output of L-asparaginase could be reached under SSF. 5 G of wheat bran were dispensed in 250 ml Erlenmeyer flasks and moistened with 5 ml 0.1 M phosphate buffer pH7.4, sterilized and mixed with 3 ml of seed culture of the tested bacterium. The enzyme activity was studied after 12, 24, 36, 48, 72, 96 hrs of incubation periods.

Waste / Buffer ratio

In the present study, the fermentation media was modified by using different waste/buffer ratio (w/v) in the following order 1:1, 1:2, 1:3, 1:4: 2:1, 2:3, 3:1, 4:1 to test the effect of moisture content on *Stenotrophomonas* sp FZL-asparaginase activity.

Effect of inoculum level

In order to test the effect of inoculum concentration on L-asparaginase production by the bacterium under test, the sterilized fermentation media dispensed in 250 ml Erlenmeyer flasks received different inoculum levels (1,3,5,7 and 9 ml) of 18 hrold cultures ($A_{550}\leq 1$) and incubated at 30˚ C ± 2 for 48 hrs.

Effect of incubation temperature

In trial to test the optimum temperature for production of L-asparaginase by the bacterium under investigation, the fermentation medium was prepared, sterilized and inoculated with 3 ml as a standard inocula and incubated at different temperatures (25, 30, 35, 40, 45 and 50 ˚ C) for 48 hrs incubation period.

Optimization of *Stenotrophomonas* sp FZL-asparaginase production using multifactorial experiments (Plackett-Burman Design)

Plackett-Burman design (Placket & Burman,1946), well established statistical technique for medium component optimizationwas applied to screen the medium components critically affecting L-asparaginase activity by *Stenotrophomonas* sp FZ.The medium components were screened for eleven variables at two levels, maximum (+) and minimum (-) . According to the Plackett-Burman design, the number of positive signs (+) is equal to (N +1)/2 and the number of negative signs (-) is equal to (N-1)/2 in a raw. A column should contain equal number of positive and negative signs. The first row contains (N+1)/2 positive signs and (N-1)/2 negative signs and the choice of placing the signs is arbitrary. The next (N-1) rows are generated
by shifting cyclically one place (N-1) times and the last row contains all the negative signs. Response is calculated as the rate of caffeine degradation expressed as percentage (%) or g/l.
The effect of each variable was calculated using the following equation:

$$E_{xi} = \frac{\sum M_{i+} - M_{i-}}{N}$$

where E_{xi} is the effect of the tested variable, M_{i+} and M_{i-} the rates of caffeine degradation from trials in which the variables being measured were added to the medium at their maximum and minimum level respectively and N is the number of experiments carried out, which is 12 in this case, as the number of variables to be tested is 11.
The standard error (S.E) of the variables was the square root of variance and the significance level (P value) of each variable is calculated by using the *t*- Test:

$$t = \frac{E_{xi}}{S.E}$$

where E_{xi} is the effect of the tested variable. The variables with higher confidence levels were considered to influence the response or output variable.

RESULTS AND DISCUSSION

Screening experiments for bacterial production of L-asparaginase

Qualitative method (Using rapid plate assay technique).

Bacterial species were isolated from the soil sample collected from Bekka valley. The isolates were screened for L-asparaginase production by plate assay. The isolate that showed pink zone around the colonies indicated L-asparaginase production and was selected for determination of enzyme activity.

Quantitative method

The fermentation process was conducted under static fermentation (SSF) and shaken (SF) conditions at 30◦C for 48 hrs.Tthe fermentation media were inoculated with 3 ml standard inocula and incubated at 30◦C for 24 hrs unless otherwise indicated.

Under submerged fermentation

Data represented in Fig.1 revealed that the selected bacterium under test was able to grow and produce L-asparaginase under submerged fermentation technique using shaken and static conditions where the enzyme yield was 0.905 and 0.780 IU respectively.

Under solid state fermentation

The bacterium under test was able to grow and produce L-asparaginase enzyme effectively (0.981 IU) under solid state fermentation. Solid state fermentation was selected for further experiments for production of L-asparaginase due to high enzyme production and its advantage over SF

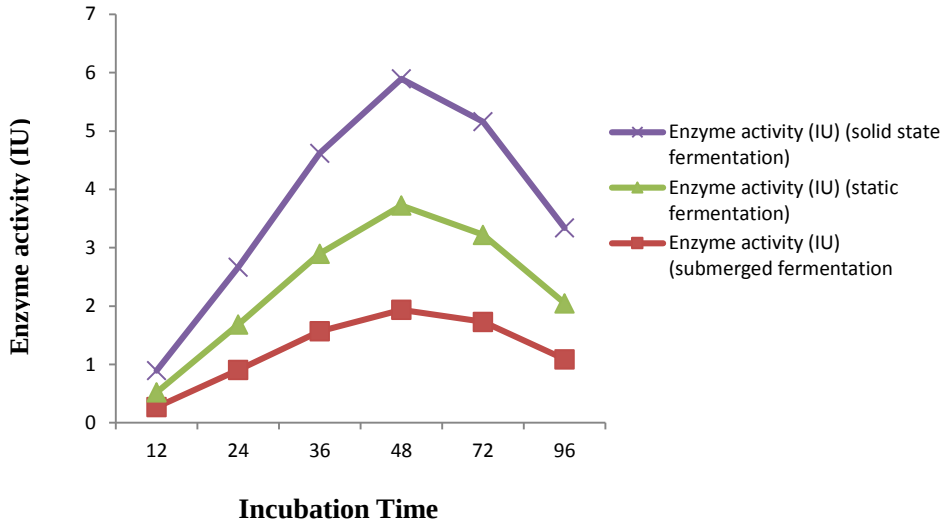

Fig1 –*Stenotrophomonas*sp FZL- asparaginase activity as affected by incubation time

Bacterial identification

Phenotypic characterization

Gram negative , rod shaped isolate was identified as *Stenotrophomonas*sp FZ Morphological observation revealed that the colony was translucent and small with raised elevation.

Genotypic characterization and phylogeny

The most promising bacterial isolate was identified by sequencing PCR amplified 16S rNDA. The obtained sequences were submitted to FASTA3 data base in order to find homologies with other 16S rDNA. Table 1, shows the similarities percentages and accession numbers obtained after comparing the sequence of the tested strain to the submitted sequences in gene bank. The tested strain was affiliated to the genus *Stenotrophomonas* with 86% similarity to*Stenotrophomonas maltophilia* R551-3 . The phylogeny of the tested strain and closely related species was analyzed using multi sequence alignment program and the results are presented in phylogenetic tree The strain showed 86% Identity to *Stenotrophomonas maltophilia* R551-3 (ac: NR074875.1) consequently the bacterial strain was named as *Stenotrophomonas*sp FZ.

Table 1- 86 % Similarity percentage scores of 16S rDNA sequences for the selected isolate compared to those obtained from database.

Isolate	Identify	Accession number	% of similarity
1	*Stenotrophomonas Maltophilia* R551-3	NR074875.1	86
2	*StenotrophomonasKoreensis* TR6-01	NR041019.1	84
3	*Lysobacter oryzae* YC6269	NR044484.1	83
4	*Pseudomonas Pictorium LMG981*	NR041957.1	84

Effect of incubation period

Figure (1) indicated that maximum enzyme activity (0.981 IU) was achieved after 48 hrs of incubation at the end of the log phase then the bacterial growth entered the stationary phase until 72 hrs, followed by a sharp decrease in the enzyme production when the bacterial growth entered the decline phase.The maximum enzyme production by *Fusariumoxysporum*wasobserved at 120 hrs (Chankaya et al., 2010) while *Penicillium* L-asparaginase was produced at 96 hrs of incubation (Soniyamby et al., 2011).Yogendra*(*2011) reported that *Bacillus aryabhattai*strain ITBHU02have maximum enzyme activity after 35 hrs (16.1 UI). Maximum enzyme activity out put of*E.coli* and *P.aeruginosa*were 0.805 UI and 0.055 UI respectively after 24 hrs of incubation (Roberts, 1968).

Waste/ Buffer ratio

In order to test the effect of moisture content on the production of L-asparaginase, the fermentation media containing 5 g wheat bran were fortified with different volumes of 0.1 M of phosphate buffer pH 7.4. Data in the present study presented in in fig (2) indicated that the maximum enzyme activity (2.320 IU) was attained with waste/ buffer moisture content of 1:1 (w/v) while low enzyme activity (1.277 IU) was attained with 2:1 (w/v). The initial moisture was 60 % (w/v) by *FusariumOxysporium*(Chankaya et al., 2010). Moreover 1:1 (w/v) initial moisture content for maximum enzyme activity by *Bacillus*sp was noticed by Yogendra (2011).Moisture content is known to be the most studied parameters in soli-state fermentation, because the growth and the metabolism of microorganisms in SSF mostly occur in the liquid phase. Optimal moisture content depends on the microorganisms and substrate. Lower moisture levels reduce the solubility of the nutrients in solid substrate, lower the degree of the substrate swelling and increase the water

tension. Similarly, higher moisture is reported to decrease porosity, alter the particle structure, develop stickiness and decrease gaseous exchange (Pandey, 2003).

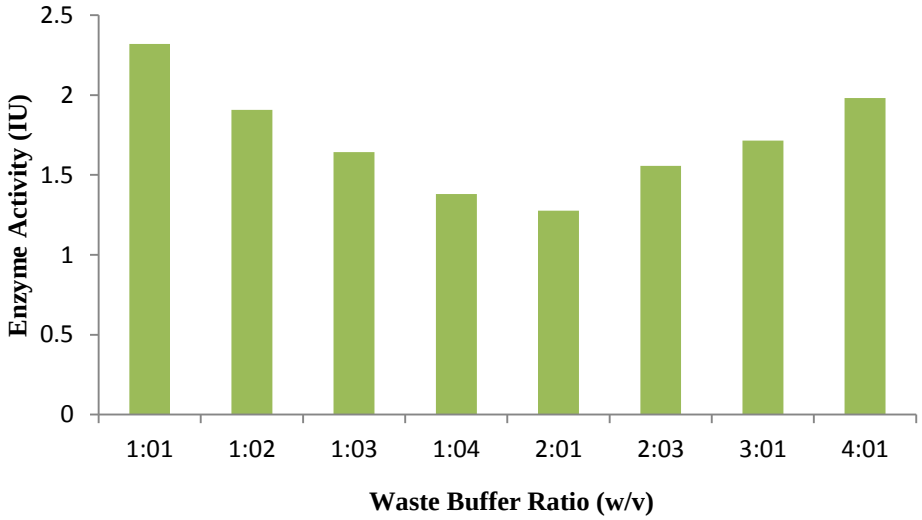

Fig 2 :*Stenotrophomonas*sp ZFL- asparaginase activity as affected by waste/buffer ratio (w/v)

Effect of inoculum level

Standardinocula of 3 ml of 18hrs old *Stenotrophomonas*sp ZF cultures ($A_{550}\leq 1$) were routinely used to inoculate the fermentation medium. The following experiment was carried out to find the optimal seed culture volume / flask ratio for L-asparaginase production. Therefore, different inocula levels (1, 3, 5, 7 and 9 ml/ flask) were used. The results shown in Fig 3 revealed that as the inoculum level increased the enzyme activity decreased. Maximum enzyme accumulation (2.430 IU) was obtained with inoculum size 3 ml/ flasks; however minimal enzyme activity (0.781 IU) was obtained with inoculum size of 9 ml/ flasks. According to these results, 3 ml inoculum level/flask was selected to carry out the next part of the research. On the other hand, Gurubasappa (2010) reported that the optimum inoculum level for L-asparaginase production was found to be 7 ml. 1.5 ml of inoculum level was found to be the optimum condition for L-asparaginase production by *FusariumOxysporium*(Chankaya et al., 2010).Yogendra, 2011foundthat 2 ml of inoculum level lead to maximum enzyme activity by *Bacillus*sp .*E.coli* sp and *P.aeruginosa* have maximum enzyme activity with 0.1 and 0.2 ml of inoculum level respectively (Roberts, 1968). A higher inoculums level increased the moisture content to a significant point and free excess liquid present in and unabsorbed form will therefore give rise to an additional diffusional barrier together with that inposed by solid nature of the substrate and lead to decrease in growth and enzyme production .

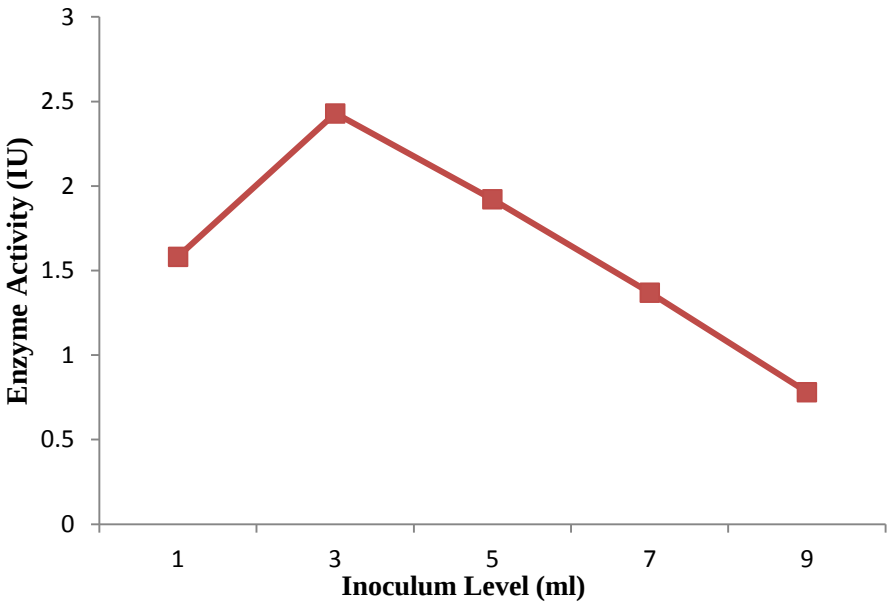

Fig 3 :*Stenotrophomonas*sp FZ L- asparaginase activity as affected by inoculum level

Effect of incubation Temperature

In trial to determine the optimum incubation temperature that leads to maximum enzyme production, the inoculated fermentation medium was incubated at different temperatures (25, 30, 35, 40, 45 and 50◦C for 48 hrs. Results in Fig (4) indicated that the maximum enzyme activity (2.328 IU) was achieved at 35◦C incubation temperature. Although, the physiological changes persuaded by high temperatures during enzyme production are not entirely understood, it has been recommended that a high temperature, microorganisms may synthesize only a reduced numbers of proteins essential for growth and other physiological processes (Yogendra, 2011). The optimum temperature production by *Aspergillusterreus*was 35◦C (Lingappa., 2010) while *Fusariumoxysporum* was produced at 30◦C (Chankaya et al., 2010). Soniyamby et al., (2011) reported that the maximum *Penicilluim* enzyme activity was found at 30◦C.

Effect of pH

The production of a L-asparaginase by microorganisms is strongly dependent upon medium pH as it plays role in transportation of various components across the cell membrane and in managing the metabolic activities of the cell (Yogendra, 2011). In order to test the L-asparaginase production against pH changes, the fermentation media were adjusted to different pH values (1, 5, 7, 9 and 12) one at a time .The media were incubated for 48 hrs at 35◦C after inoculation with 3 ml/flask of bacterial suspension as standard inoculum. Results presented in Fig (5) revealed that the optimum

pH for the maximum production of the enzyme was pH 7 achieving 2.128 IU enzyme activity. As the pH moves towards being acidic or basic, the production of the enzyme decreases.*Fusariumoxysporum*grew effectively at pH range of 4-7, and the maximum enzyme production was noticed at pH 7 (Chankaya et al., 2010). Maximum L-asparaginase activity was exhibited at pH 4.5 by *Aspergillusterreus*(Lingappa., 2010). The optimum pH for enzyme production by *Streptomyces Albidoflavus*was 8 (Narayana and Vijayalakshmi, 2008).

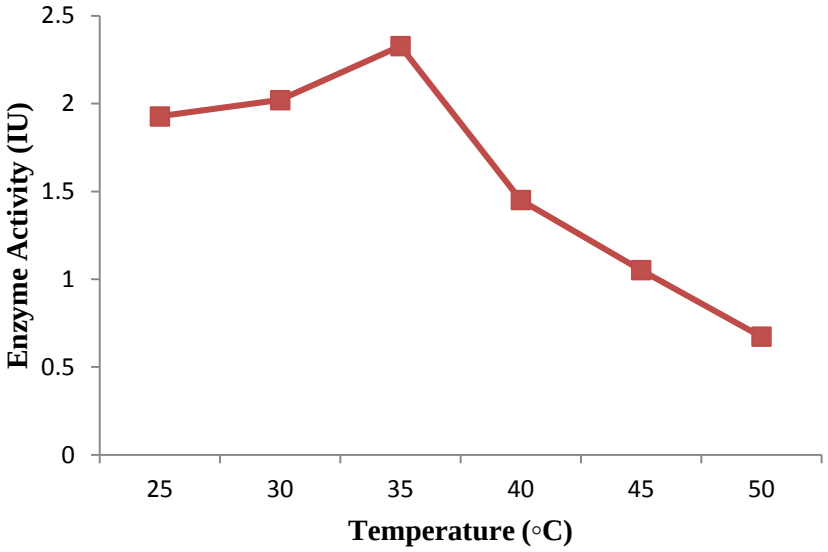

Fig4 :*Stenotrophomonas*sp ZF L- asparaginase activity as affected by incubation temperature

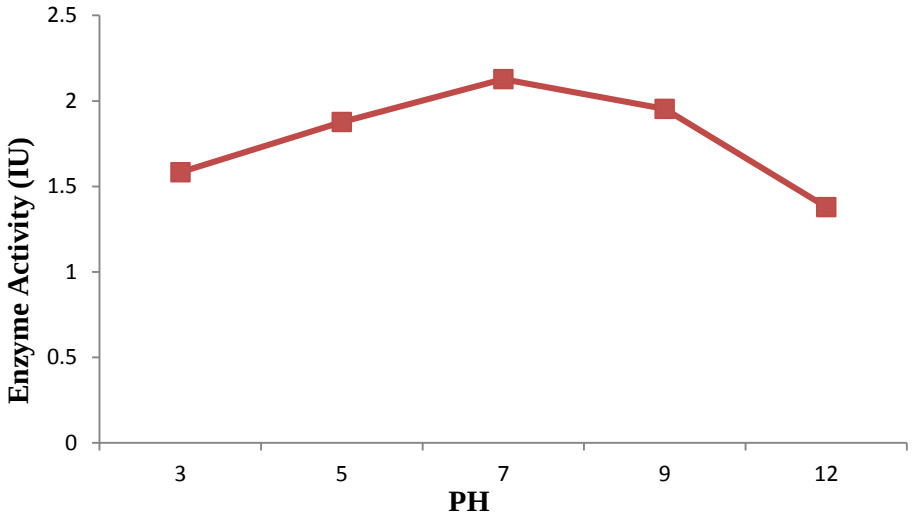

Fig 5: *Stenotrophomonas*sp ZF L- asparaginase activity as affected by pH

Optimization of the nutritional factors affecting L-asparaginase production using multifactorial statistical designs: Plackett- Burman design

In the first approach, the placket-burman design was applied to reflect the relative importance of various nutritional factors involved in L-asparaginase production. Eleven different factors (variables) were chosen to perform this optimization process. The independent variables were examined and their settings are shown in table 2.At the end of incubation time, the enzymes activity was measured.All experimental conditions (trials) were performed in duplicate. The results are given in table 3. The main effect (that was estimated as a difference between both average measurements made at high level (+) and at the low level (-) of the factor) of the examined factors affecting L-asparaginase production wascalculated and presented graphically (Fig. 6). On the analysis of the regression coefficients of the eleven variables glucose, casein, asparagine, zinc sulfate, potassium chloride and magnesiumhad shown a positive effect whereas maltose, ammonium sulfate, urea , sodium nitrate and ferrous sulfate had shown a negative effect.At the end of incubation time, the enzymes activity was measured.All experimental conditions (trials) were performed in duplicate. The results are given in table 3. The main effect (that was estimated as a difference between both average measurements made at high level (+) and at the low level (-) of the factor) of the examined factors affecting L-asparaginase production wascalculated and presented graphically (Fig. 6). On the analysis of the regression coefficients of the eleven variables glucose, casein, asparagine, zinc sulfate, potassium chloride and magnesiumhad shown a positive effect whereas maltose, ammonium sulfate, urea , sodium nitrate and ferrous sulfate had shown a negative effect

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Table 2 : Variables and their levels employed in the Plackett-Burman design for screening of nutritional conditions affecting *Stenotrophomonas* sp ZF L-asparaginase production

Variables	Code	Low level (-1)	Basal medium	High level (+1)
Glucose	Glu	10	20	30
Maltose	Mal	10	20	30
Casein	Cas	10	20	30
Ammonium sulfate	AmS	10	20	30
Urea	Ur	10	20	30
Sodium nitrate	SD	10	20	30
Asparagine	Asp	0	2	4
Zinc Sulfate $ZnSO_4$	$ZnSO_4$	0.20	0.28	0.36
Potassium chloride	KCl	0.04	0.16	0.10
Ferrous sulfate	$FeSO_4$	0.20	0.28	0.36
Magnesium sulfate	Mg SO_4	0.06	0.12	0.18

Statistical analysis of the data is demonstrated in table 4 as t-value for the eleven experimental variables. The significance level was determined using the student's test. The t-test for any individual effect allows an evaluation of the probability of finding the observed effect purely by chance.If the probability is sufficiently small, the idea is that the effect was caused by varying the level of the variables under test is accepted. Confidence level is an expressiom of the P-value in percent.The factors whichshowed a high confidence percentage are initial casein and magnesium sulfate where they are the most positive significant variables affecting L-asparaginase production (Fig.7).Ammonium sulfate was the most negative significant variables affecting the asparaginase production especially in trials 5, 9 and 10 where the enzyme activity was 6.25, 7.20 and 7.9 IU respectively. On the other hand, the L-asparaginase activity was the highest in the absence of this element in trial 1 with 21.23 (IU) of enzyme activity. However casein was the most positive

significant variable affecting the asparaginase production under solid state fermentation. It seems to affect negatively the asparaginase production in trials 2,5,9 and 10 where the enzyme activity was 7.11, 6.25, 7.20 and 7.00 IU respectively. On the other hand, the L-asparaginase activity was the highest in the presence of this element in trials 1,3,6 and 8 (21.23, 11.25, 8.65 and 6.25 IU respectively).

Table 3: Randomized Plackett-Burman experimental design for evaluating factors influencing L- asparaginase production by *Stenotrophomonas* sp ZF under solid state fermentation

Variables Trials	Glu	Asp	Cas	KCl	NH_4 SO4.	$NaNO_3$	$ZnSO_4$	$FeSO_4$.	Ur	Mal	Mg SO_4	Asparaginase production
1	+	-	30	-	-	-	+	+	+	-	0.18	21.23
2	+	+	10	+	-	-	-	+	+	+	0.09	7.11
3	-	+	30	-	+	-	-	-	+	+	0.18	11.25
4	+	-	30	+	-	+	-	-	-	+	0.18	7.96
5	+	+	10	+	+	-	+	-	-	-	0.18	6.52
6	+	+	30	-	+	+	-	+	-	-	0.09	8.65
7	-	+	30	+	-	+	+	-	+	-	0.09	6.09
8	-	-	30	+	+	-	+	+	-	+	0.09	8.25
9	-	-	10	+	+	+	-	+	+	-	0.18	7.20
10	+	-	10	-	+	+	+	-	+	+	0.09	7.90
11	-	+	10	-	-	+	+	+	-	+	0.18	7.00
12	-	-	10	-	-	-	-	-	-	-	0.09	8.36
13	0	0	0	0	0	0	0	0	0	0	0	6.8

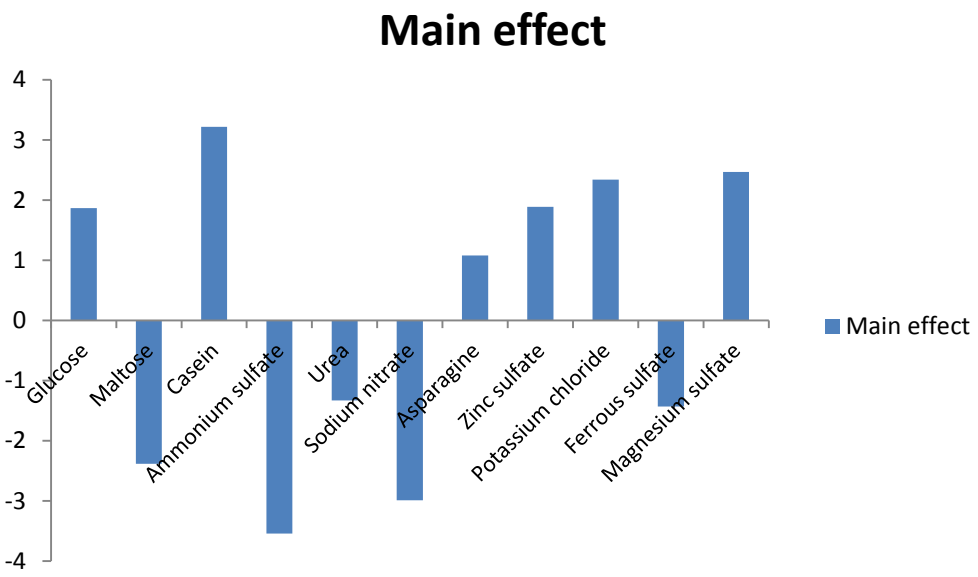

Fig 6: Main effect of variables on L-asparaginase production by *Stenotrophomonas* sp ZFunder shaken conditions

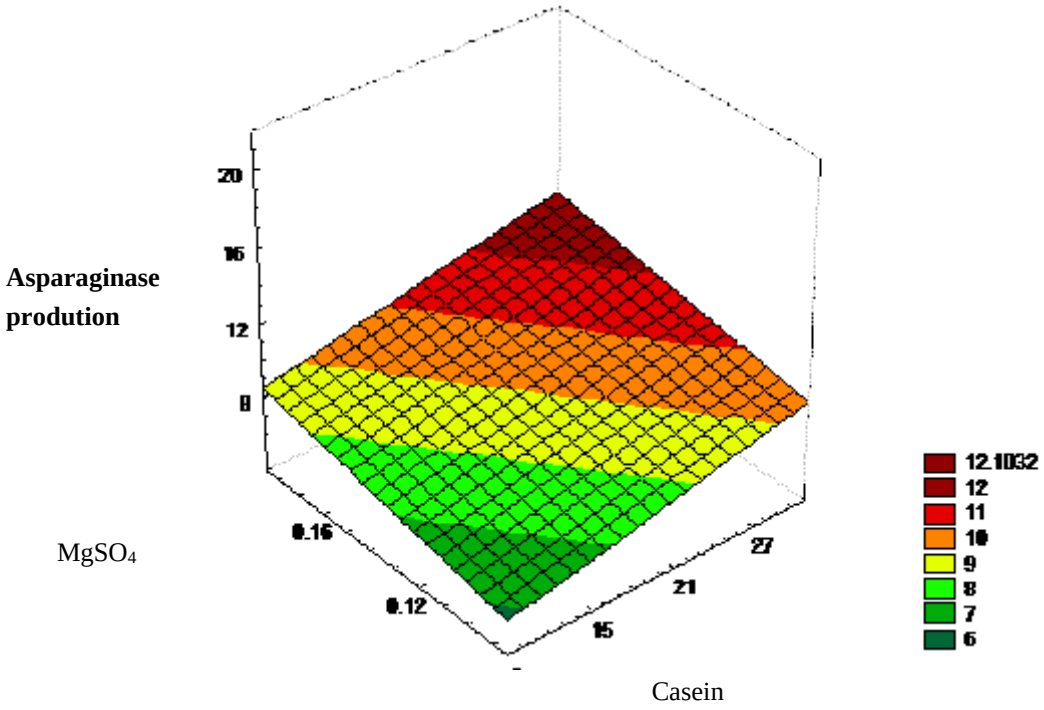

Fig 7: Effect of casein and magnesium sulfate concentration on L-asparaganise production by *Stenotrophomonas*sp ZF

Table 4 : Statistical analysis of Plackett-Burman experiment for *Stenotrophomonas*sp ZF L-asparaginase production

Variables	Symbols	Main effect	t-test
Glucose	Glu	1.87	0.343828
Maltose	Mal	-2.38	-1.01044
Casein	Cas	3.22	2.015048
Ammonium sulfate	AmS	-3.54	-1.60747
Urea	Ur	-1.33	-0.54582
Sodium nitrate	SD	-2.99	-1.30693
Asparagine	Asp	1.08	0.439602
Zinc sulfate	$ZnSO_4$	1.89	0.789166
Potassium chloride	KCl	2.34	0.991768
Ferrous sulfate	FeSO4	-1.43	-0.58823
Magnesium sulfate	MgSO4	2.47	2.570582

CONCLUSION

A novel bacterialstrain namely :*Stenotrophomonas*sp ZF was isolated from Lebanese soil and can be considered as a candidate for L- asparaginase production in large scale industry to be used in the treatment of lymphoblastic leukemia.

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