JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

T.A. Thaer & P. Ellaiah. L-Asparaginase production by a streptomycete and optimization of production parameters. Journal of pharmaceutical and biomedical sciences (J Pharm Biomed Sci.) 2013, April; 29(29): 859-869. (Article no 24)

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Journal of Pharmaceutical and Biomedical Sciences (J Pharm Biomed Sci.), Member journal. Committee of Publication ethics (COPE) and Journal donation project (JDP).

PBMS
Journal of Pharmaceutical and Biomedical Sciences
ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.
T.A. Thaer & P. Ellaiah. J Pharm Biomed Sci. 2013, April; 29(29): 859-869.
Available at jpbms.info

Research article

L-Asparaginase production by a streptomycete and optimization of production parameters

T.A. Thaer * and P. Ellaiah

College of Pharmaceutical Sciences, Andhra University, Visakhapatnam -530003
A.P., India.
* Present Address: Department of Medical Lab Sciences, Faculty of Allied Medical Sciences, Arab American University, P.O. Box 240, Jenin, Palestine.

Abstract :
A **Streptomycete sp** (A2') was selected as the best L-asparaginase producing strain among (53) isolates. The fermentations were conducted by shake flask. Among carbon sources used mannitol was found to be the best for L-asparaginase production, its optimum concentration being 0.1%. Casein and corn steep liquor were preferred nitrogen sources for L-asparaginase production, with optimum concentration of casein being 0.75 %. The optimum pH range for L-asparaginase production was 6.5 to 7.5, optimum being 7.0. The optimum incubation temperature was 28°C. The maximum production attained at 72 hours of incubation. A 10% level of inoculum was found to be optimal for L-asparaginase production. At ratio 1:10 v/v (Volume of medium per volume of flask), the activity of L-asparaginase was maximum. Agitation was shown to have influence on L-asparaginase production. L-asparagine substrate was found to be a good enzyme inducer. The culture conditions of the strain for L-asparaginase production were optimized. The maximum production rate of L-asparaginase was improved 3 folds under the optimal conditions.

Key Words: Marine **Actinomycetes**, **Streptomyces**, L-asparaginase, production, optimization.

Introduction:

Marine microbes represent a potential source for commercially important bioactive compounds. Among marine microorganisms, actinomycetes have gained special importance as the most potent source of antibiotics and other bioactive secondary metabolites[1,2]. Marine actinomycetes are a prolific source of secondary metabolites and the vast majority of these compounds are derived from the single genus **Streptomyces**[3]. **Streptomyces** species are distributed widely in marine and terrestrial habitats and are of commercial interest due to their unique capacity to produce novel metabolites[4]. While most of the studies on actinomycetes have focused on antibiotic production, only few reports have dwelt on their enzymatic potential[5]. Marine actinomycetes particularly **Streptomyces** may be a potential source L-asparaginase[2,6-8]. Several terrestrial **Streptomyces** are capable of producing detectable amount of L-asparaginase[9]. There are limited reports on production of L-asparaginase from marine **Streptomyces**[2,8,10].

Marine actinomycetes have been reported to produce functionally unique metabolites and enzymes that are not found in their terrestrial counterparts. This is due to their extreme living conditions within the marine environment. Recent studies have shown that few antitumour compounds isolated from marine actinomycetes are under clinical trials[11]. Hence, as a new source of antitumour drug candidates, marine actinomycetes have attracted serious attention in the last decade.

L-asparaginase enzyme (E.C.3.5.1.1) is an anti-neoplastic agent used in the lymphoblastic leukaemia chemotherapy[12,13]. L-asparaginase today is regarded as one of the useful components of the antitumor armamentarium. It has received increased attention in recent years for its anticarcinogenic potential. The enzyme L-asparaginase has been intensively investigated over the past two decades owing to its importance as anti neoplastic agent[14]. For these reasons L-asparaginase has established itself to be an indispensable component[15]. This treatment brought a major breakthrough in modern oncology, as it induces complete remission in over 90% of children within 4 weeks[16]. L-asparaginase is also used commercially to reduce the formation of acrylamide in fried foods[17].

Microbes are the better source of L-asparaginase, because they can

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Available at jpbms.info

be cultured easily and the extraction and the purification of L-asparaginase from them are also convenient, facilitating the large scale production[18]. Literature reports indicated that the enzyme's biochemical and kinetic properties vary with the genetic nature of the microbial strain used[19]. This may require the screening of different samples from various sources for isolation of potential microbes, which have the ability to produce the desired enzyme. Recently, Gupta et al. 2007[10] reported that marine actinomycetes have been shown to be a good source for L-asparaginase because of extreme adaptation within the marine environment. Commercially used enzymes are obtained from E. coli and Erwinia carotoiora[2,20]. Therefore there is a continuing need to screen newer organisms in order to obtain strains capable of producing new and high yield of L-asparaginase[12,21]. The oceans around India remain an untapped source of many drugs and several studies indicate that many pharmacologically active substances can be isolated from marine organisms[22]. Thus; there is enormous scope for investigations to explore the possibilities of deriving new products of economic importance from potential marine microorganisms. The enzyme is produced throughout the world by both submerged and solid-state cultures[6].

In this article, attempts were made on the production of an extra-cellular L-asparaginase from marine actinomycetes particularly Streptomyces under submerged fermentation.

Materials and methods:

Microorganism and cultivation conditions:

The isolates used in the present study were 53 actinomycetes which were isolated from soil samples and marine substrates in and around Viskhapatnam, India. Actinomycetes were subcultured on starch casein agar and jowar starch medium slants and incubated for 7-10 days at 28° C, then they maintained at 4°C until further use. A marine Streptomyces sp (A2'), isolated from sea sediment collected from Viskhapatnam at our laboratory was used in the present study[23]. The organism was maintained on starch casein agar and jowar starch medium slants incubated at 28°C for 7 days. Inoculum was prepared from 7 day old culture of the strain A2'.

Screening of isolates for L-asparaginase production:

The isolates (53) were screened for L-asparaginase activity qualitatively using the method of[24]. The medium used was incorporated with L-asparagine and pH indicator (phenol red). L-asparaginase activity was identified by change of color from yellow to pink (red) color.

Production of L-asparaginase:

Tryptone glucose yeast extract medium (TGY) was used for production. The medium consisted of (glucose, l.0 g; K_2MPH_4, 1.0 g ; yeast extract, 5.0 g; tryptone, 5.0 g; water 1.0 liter, pH adjusted to 7.0). A 25 ml of broth in 250 ml EM flasks were inoculated and incubated on rotary shaker (220 rpm) at 28°C for 72 hr. Samples were withdrawn at regular interval of time and measured for L-asparaginase production. Experiments were conducted in triplicates, and enzymatic assay was performed in triplicates for each sample.

Assay of L-asparaginase:

At the end of the incubation period, 10 ml of the cell suspension was centrifuged and the cell mass was washed thrice with 0.15 M NaCl containing l0 mM (EDTA). The cell mass was taken up in 5 ml of water and sonicated by agitating the suspension with glass beads on rotary shaker for 1 hr. One ml of the cell suspension was taken and added to 2 ml of l0 mM L-asparagine in 0.05 M tris buffer pH7.2 containing 0.1 mM EDTA. The cell suspension was analyzed for intracellular L-asparaginase activity. The activity of L-asparaginase was measured by modified Nessler's method described by[25].One international unit (IU) of L-Asparaginase is that amount of enzyme which liberates 1μM of ammonia in 1 min. at 37°C.

Media optimization:

The choice of medium is virtually as important to the success of an industrial fermentation as the selection of organism to carry out the fermentation[26]. The medium requires the selection of carbon, nitrogen and inorganic salts, as well as energy sources that will support not only good microbial growth but also maximize the product yield, minimize the synthesis of compounds closely related to the product, and enhance product recovery. Different parameters were used to study their effect on growth and enzyme production. Medium was supplemented with different carbon and nitrogen sources with different concentrations to study their effect on growth and enzyme production. The effect of cultural conditions like different

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initial pH, incubation temperature, incubation period, level of inoculum, aeration, agitation, L-asparagine substrate on growth and enzyme production was studied. Finally production of enzyme with the optimum conditions (using modified production medium and cultural conditions formulated based on the above observations) was studied.

It was also compared with that of bacterial strains B-26 and Erwinia carotovora. The B-26 is a bacterial species isolated in our laboratory and Erwinia carotovora ATCC 15713 is an L-asparaginase producing reference culture obtained from NCL, Pune, India.

Results:

The isolates used in the present study were 53 actinomycetes isolated from soil samples and marine substrates in and around Viskhapatnam, India. These were screened qualitatively for their L-asparaginase activity[24]. The L-asparaginase active isolates (48) were subjected to quantitative analysis by direct Nesslerizalion method[25]. The isolate Streptomyces strain A2' (from marine sample) exhibited excellent activity was selected for detailed optimization studies.

Effect of different carbon sources on L-asparaginase production:

To study the effect of various carbon sources on L- asparaginase production, different carbon sources were tried in the basal production medium (without glucose). Glucose (0.1%) was replaced in the production medium by various carbon sources i.e. soluble starch, tapioca starch, sucrose, mannitol, fructose, lactose, and maltose. The relative yields of L-asparaginase are shown in Figure 1.

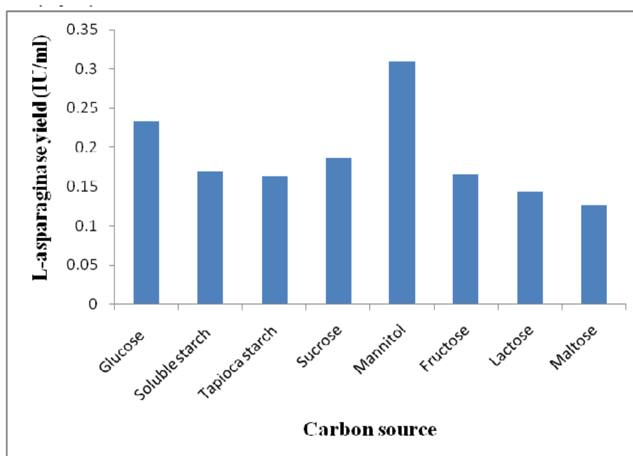

Figure 1. Effect of different carbon sources on *L-asparaginase* production

Among the various carbon sources investigated, mannitol was found to be the best carbon source for L- asparaginase production. So, mannitol was selected for further optimization studies for L- asparaginase production.

Effect of different concentrations of mannitol on L-asparaginase production:

The effects of different concentrations of mannitol were investigated to study their effect on L-asparaginase production. The results are given in Fig. 2. Mannitol at a concentration of 0.1 (w/v) gave maximum L- asparaginase production.

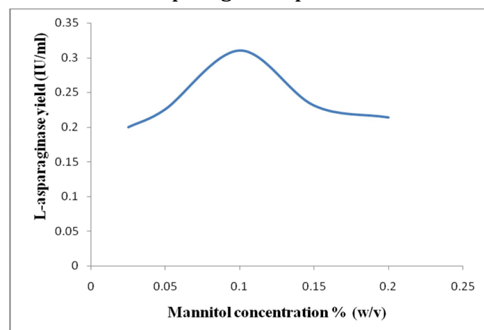

Figure 2. Effect of different concentrations of mannitol on L-asparaginase production

Effect of various nitrogen sources on L-asparaginase production:

Tryptone in the production medium containing mannitol as carbon source was replaced with different nitrogen sources to study their effect on L- asparaginase production. The results are presented in Fig. 3.The results indicated that casein is the best nitrogen source for L-asparaginase production followed by corn steep liquor.

Effect of different concentrations of casein on L-asparaginase production:

Various concentrations of casein were tried to study their effect on L- asparaginase production. The results are given in Figure 4. Casein at a concentration of 0.75 % (w/v) gave maximum L- asparaginase production.

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ISSN NO- 2230 – 7885
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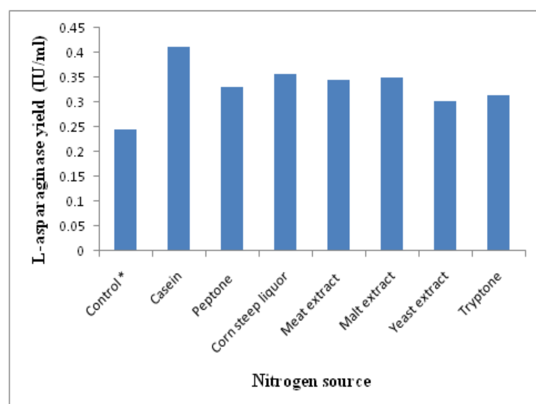

Figure 3. Effect of various nitrogen sources on L-asparaginase production

* Medium without tryptone and yeast extract

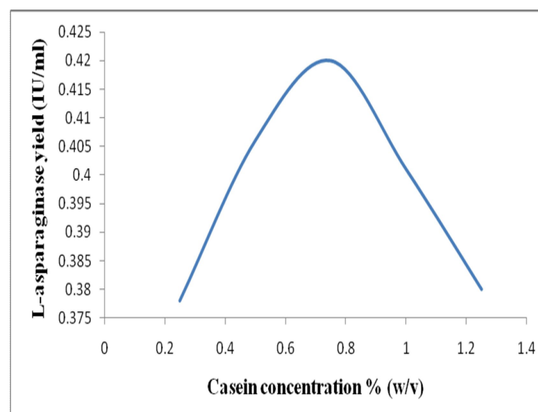

Figure 4. Effect of different concentrations of casein on L-asparaginase production

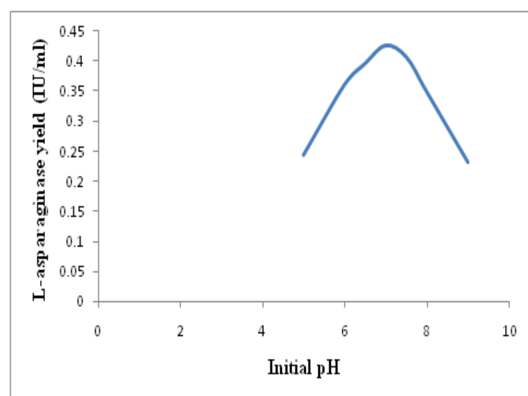

Figure 5. Effect of initial pH on *L- asparaginase* production

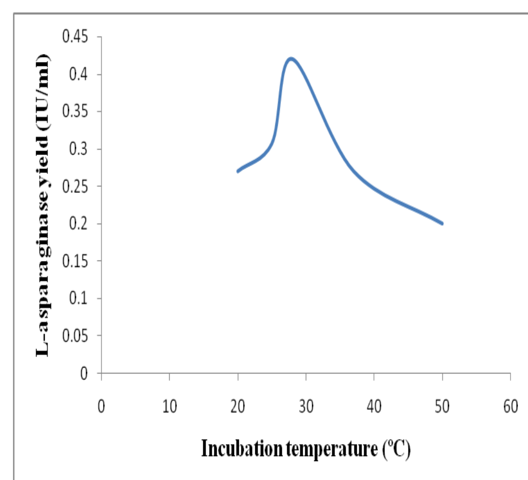

Figure 6. Effect of incubation temperature on *L-asparaginase* production

Effect of initial pH on L- asparaginase production:

The effect of initial pH on the L-asparaginase production was tested in the range of 5.0 to 9.0. The results are given in Fig. 5. The optimum pH for L-asparaginase production was found to be 7.0.

Effect of incubation temperature on L-asparaginase production:

The effect of incubation temperature on the production of L-asparaginase was studied in the range of 25°C to 50°C. The L-asparaginase production was found to be maximum at 28°C (Figure 6). Drastic decrease in L-asparaginase production was observed at higher temperatures.

Effect of incubation period on L-asparaginase production:

To determine the effect of incubation period on L-asparaginase production, different incubation periods were tried. The results are shown in Fig. 7. An incubation period of 72 h was found to be optimal for maximum L- asparaginase production after which the productivity was gradually decreased.

Effect of Level of inoculum on L-asparaginase production:

To study the effect of level of inoculum on L-asparaginase production, different levels of inocula were tried. Different inocula levels varied from 5.0 to 15 % (v/v) were studied. The results are shown in Table 1. Inoculum level of 10 % gave maximum L-asparaginase production.

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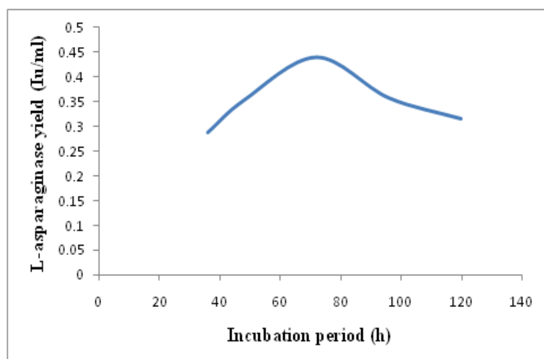

Figure 7. Effect of incubation period on L-asparaginase production

Table 1. Effect of Level of inoculum on L-asparaginase production

Level of inoculum in % (v/v)	L- asparaginase yield (IU/ml)
5.0%	0.345
10.0%	0.420
15.0%	0.366

Effect of aeration on L-asparaginase production:

The effect of aeration on the production of L-asparaginase was studied by varying the medium volume in the production flasks and results are shown in Table 2. At ratio of 1:10 V/V (volume of medium per volume of flask), the activity of L-asparaginase was maximum. Decrease in activity was noticed when volume of the medium was increased.

Table 2. Effect of aeration on L-asparaginase production

Volume of medium (ml)	L- asparaginase yield (IU/ml)
12.5	0.385
25.0	0.425
37.5	0.373
50.0	0.257

Effect of agitation on L-asparaginase production:

In order to estimate the effect of agitation on antifungal production, different types of flasks indented (bottom and side) with different capacities were tried. The results are shown in Table 3. The indented bottom flask showed maximum L-asparaginase production.

Table 3. Effect of agitation on L-asparaginase production

Type of flask	L- asparaginase yield (IU/ml)
Un-indented	0.422
Side indented	0.434
Bottom indented	0.448

Effect of L-asparagine substrate on L-asparaginase production:

The effect of L-asparaginase as a substrate was studied with our strain. The L-asparagine was incorporated into the production medium at 0.2% level. Enhanced yield of L-asparaginase was observed.

L-asparaginase production using all optimum parameters:

An attempt was made to evaluate the extent of improvement in the modified formulated production medium. L-asparaginase production was tested by our isolate A2' employing the modified medium and optimized cultural conditions using all the parameters described previously. The results are presented in Figuer 8. Significant improvement (3 folds) in the L- asparaginase production was observed with the modified production medium.

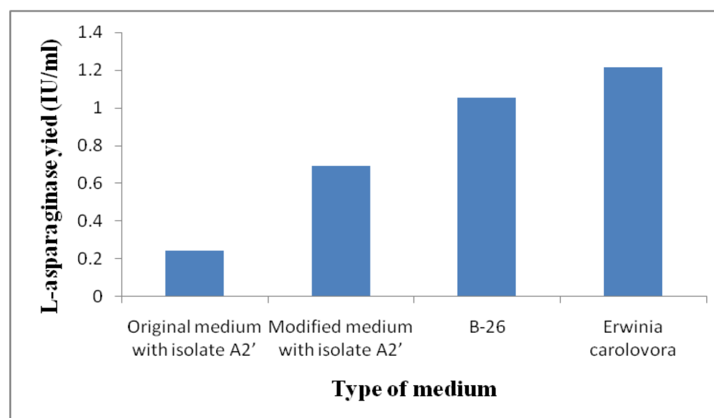

Figure 8. L-asparaginase production using all optimum parameters

It was then compared with that of bacterial strains B-26 and Erwinia carotovora. The results are shown in Figure 8. The results indicated that the bacterial isolate has comparative yield of L-asparaginase with much studied Erwinia carotovora species. However, our isolate produced reasonably good amount of L-asparaginase

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Available at jpbms.info

Discussion:

Actinomycetes perform significant biogeochemical roles in nature and are highly valued for their unparallel ability to produce wide variety of biologically active secondary metabolites[27]. Actinomycetes are able to utilize a great variety of organic compounds as sources of energy[28]. Although L-asparaginase from bacteria has been extensively characterized, a similar attention has not been paid to actinomycetes[8].

The literature indicated that yield is not only dependent on the nature of the strain and composition of medium but also on the cultural conditions[29]. Nutritional manipulation enhances the positive regulatory mechanisms of the production strains during cultivation[30]. The growth medium plays a very important role in the production of microbial metabolites under different conditions. Culture medium is significantly modulates the production of metabolites[31]. The nutritional sources like carbon and nitrogen, as well as the environmental factors such as incubation period, pH and temperature are known to have a profound effect on metabolites production by actinomycetes[32]. Optimization of culture conditions is essential to get high yields of the metabolites. Fermentation factors and their concentrations were important in achieving better enzyme production[33]. Such variation was also noticed with enzyme production by other microbes[34,35]. Biological production of any enzyme is a highly complex process, which involves several catalytic reactions and regulatory parameters at environmental, biochemical and genetic level[35-37].

To maximize any product / enzyme production by isolated microbial strain, the basic need is to have preliminary information on growth conditions and its associated enzyme production characteristics. Microorganisms utilize various substrates as nutrient source for growth and metabolism and subsequently produce metabolism related products. Balancing of fermentation factors with minimum experimentation to optimize enzyme production is a fine art in microbial metabolism[38]. Growth and metabolites production on synthetic media were found to be unsatisfactory[39]. Consequently we used a complex medium with organic nitrogen source, which supports both, growth and antifungal productivity.

The effects of certain nutrients on L-asparaginase production by Streptomyces strain no. A2' in submerged batch culture were studied.

Generally a quickly metabolized substance like glucose is responsible for catabolism repression but in some cases it is also reported to enhance metabolite production[12]. Different carbon sources (all used in equal concentrations of 0.1%) were tested in growth experiments for their ability to support L-asparaginase production by Streptomyces strain no. A2'. The results are given in Figure 1. The microorganism was able to grow in all the tested carbon sources. Among the wide variety of carbon sources tested, mannitol, followed by glucose proved to be the most suitable for L-asparaginase production. Enhancement of L-asparaginase production by glucose was observed in Aeromonas sp.[40]. In contrast, other investigators indicated that among several carbon sources tested, maltose proved to be the best for L-asparaginase production by different Streptomycete [9,12,38]. The highest titers of L-asparaginase production and high yields of biomass were obtained when mannitol was added to the production medium. The cultivation medium supplemented with mannitol was thus employed in all further experiments.

The effect of carbon source on growth and metabolites production is dependent upon several factors such as carbon concentration[41]. The effect of various concentrations of mannitol, varied from 0.025 % to 0.20 % on L-asparaginase production was studied. The results are shown in Figure 2. The results showed that both volumetric and specific production of L-asparaginase continues to increase and reached a maximal value at 0.10 %, above this concentration, the L-asparaginase production decreased. As mannitol concentration increased from 0.10 % to 0.20 % the L-asparaginase production decreased. On the other hand, mannitol was completely consumed when used at a concentration of 0.10 % or lower. The increase in mannitol concentration above this level resulted in the accumulation of mannitol in the cultivation medium and the remained amount depended on the initial concentration. Therefore, mannitol in a concentration of 0.10 % was used in the subsequent experiments.

The L-asparaginase activity was maximums when strain CMU-H002 was cultivated in asparagines dextrose salts broth amended with soluble starch (0.2%)[7]. The L-asparaginase activity was maximums when 0.2% glucose was used as a

carbon source [42]. Optimal yields of L-asparaginase were achieved in the medium amended with 2% glycerol, whereas its biosynthesis greatly declined with further hike in carbon source[43]. The acidic nature of fermentation medium could inhibit L-asparaginase biosynthesis and growth of actinomycetes[6]. Acidity of fermentation medium could inhibit the production of L-asparaginase synthesis[43]. High concentration of sugar is generally considered as repressor of secondary metabolisms and maximum cell growth rates can inhibit metabolites production [12]. These results are in agreement with our results.

The activity of the metabolites also varied with changes in nitrogen source[39,44]. Nitrogen source exhibited a significant effect on the metabolites production[45,46]. The effect of nitrogen compounds on the production of L-asparaginase by the strain was studied by incorporating different nitrogen sources to production medium. L-asparaginase production by the strain varied with different nitrogen compounds tested (Figure 3). The microorganism was able to grow on all nitrogen sources tested. Among the wide variety of nitrogen sources tested, casein followed by corn steep liquor proved to be the most suitable for L-asparaginase production. Lian-Xiang et al. 2003[44] showed that the highest metabolite production was achieved in the medium containing Corn steep liquor (CSL). Corn steep liquor was reported to exhibit significant stimulation of the L- asparaginase production[47]. These results are in agreement with our results. In contrast, it was indicated that the highest L-asparaginase production was obtained when yeast extract (1.5%) used as nitrogen source[7,9,43]. Also, tryptone was found to be the best medium for the L-asparaginase production[14].

It is well known that changes in the kind and concentration of nitrogen source influence greatly L-asparaginase production[46]. The results also showed that the concentration of casein (Fig. 4) greatly influenced the production of the L-asparaginase with maximum L-asparaginase yield being obtained in cultures supplemented with 0.75 % of casein. A gradual decline in L-asparaginase production was found with further rise in casein. Casein is essential for cell growth and L-asparaginase synthesis, but, in high concentrations, it inhibits the production of L-asparaginase. This might be due to the presence of high substrate concentration and induction of proteolytic enzymes[9].

The influence of medium composition, incubation temperatures, and initial pH on microbial growth and L-asparaginase production was also reported in various Streptomyces strains[7,9,38,43,48].The production and activity of L-asparaginase vary at different pH levels. Changes in external pH affect many cellular processes such as the regulation of the biosynthesis of secondary metabolites. The importance of pH for L-asparaginase production by Streptomyces was reported by several investigators who observed that the optimum pH for L-asparaginase production range between 7.0 and 8.5[9,43].The yield of L-asparaginase was maximum when pH was maintained at 7.0 (Figure 5). Increasing the medium pH led to an increase in the L-asparaginase production up to a certain limit above which any increase in the pH value was accompanied by a decrease in the L-asparaginase production and activity. This is in agreement with the results obtained by other researchers[7,38,43,48]. The intracellular secretion of microbial cells or intracellular enzymes released after the rupture of the cells could be the reason for the peak enzyme activity at pH 7. Sahu et al. 2007[48] indicated that the growth of actinomycetes and the enzyme activity are pH dependent.

Impact of incubation temperature on microbial growth and L-asparaginase production was also reported in various Streptomyces strains[7,9,43,48]. The production and activity of L-asparaginase vary at different incubation temperature. The L-asparaginase production was found to be maximum at 28°C (Figure 6). Drastic decrease in enzyme production was observed at higher temperatures. As the organism was isolated from marine source, study of the influence of temperature on the enzyme activity and growth of actinomycetes is imperative[49]. Their adaptation to the aquatic environment was probably due to the metabolic changes and the production of L-asparaginase may be unique[50]. The optimum temperature for L-asparaginase production range between 27°C and 37°C[9,43,48]. Extreme temperature did not favored cell growth or L-asparaginase production in this strain. Extreme pH and temperature did not favor cell growth as well as L-asparaginase production from S. albidoflavus[9]. This is in agreement with our results.

As mentioned earlier the medium composition, the environmental factors like incubation temperatures, and initial pH have profound influence on microbial growth and L-asparaginase production and was also reported in various Streptomyces strains[7,9,38,43,48]. Incubation period and temperature are essential factors that modulate

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ISSN NO- 2230 – 7885
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growth and significantly affect the amounts of metabolites produced. The condition of incubation influenced quantitatively the biosynthesis of metabolites as well as biomass reported

Studies carried out by some investigators revealed that maximal production L-asparaginase substances occurred after 72 h[9,38,43]. This is in agreement with our results (Figuer 7). In contrast, the highest biomass and L-asparaginase activity was observed at an incubation time of 168 h by some other investigators[7].In our case maximum L-asparaginase metabolite production was took place at the end of the exponential growth phase indicating that metabolite production was directly proportional to the growth rate[51]. It is reported that L-asparaginase production usually occurs in stationary phase. Maximum production of metabolite was achieved at the end of the exponential growth phase, indicating that the metabolite production was directly proportional to the growth rate which remained constant during stationery phase. Productivity of L-asparaginase was higher due to short duration of fermentation time than reported values (168 h)[42]. This study indicates that the large amount of the enzyme with higher activity would obtain in a short period under optimal conditions. The L-asparaginase production declined indicating its accumulation after a certain period of growth.

Optimization of inoculum size is necessary because too few spores lead to insufficient biomass, whereas too many spores lead to overproduction of biomass resulting in quick depletion of nutrients [12]. Prakasham et al. 2006[52], concluded that incubation temperature, inoculum level and medium pH, among all fermentation factors, were major influential parameters at their individual level, and contributed to more than 60% of total L-asparaginase production. The inoculum age and density markedly influence the productivity and economics of bioprocesses[53]. The maximum yield of L-asparaginase by our isolate was obtained when inoculum level of 10 % was used (Table 1). Our result is in agreement with the results obtained by other investigators for other metabolites[54,55]. In contrast, it was shown that optimal L-asparaginase production was achieved with inoculum size of 2.61% (v/v)[42].

Agitation affects aeration and mixing of the nutrients in the fermentation medium[31]. Adequate agitation was found to increase metabolite production[56]. Yegneswarant et al. 1991[57] indicated that the most effective control strategy was to control dissolved oxygen only during active growth when the biosynthetic enzymes were probably synthesized. Lian-Xiang et al. 2003[44] indicated that improvement of dissolved oxygen tension was favorable for metabolite production and pellets formation. Several techniques have been used to control dissolved oxygen in fermentations, the most common being the use of agitation speed and the aeration rate to the fermentor[57]. The yield of enzyme increased with increase in agitation speed from 140-200 rev/min and decreased later[12]. Maximum L-asparaginase production was obtained at an agitation speed of 200 rev/min and lowest yield at140 rev/min.

The L-asparaginase activity was maximums when organism was cultivated in asparagines dextrose salts broth pH 7.0 and incubated at 30ºC with shaking at 125 rpm for 7 days[7]. Aeration and agitation were most significant at interactive level, but least significant at individual level, and showed maximum severity index and vice versa at enzyme production[55]. The individual optimum level of rpm of shaking incubator was found to be 157 rpm for the production of L-asparaginase[42]. High agitation speed (220 and 260 rpm) has negative effect on the L-asparaginase production, which inferred with the production of L-asparaginase by P. carotovorum MTCC 1428 favor at low agitation speed. Geckil and Gencer 2004[58] were also observed the similar effect on the production of L-asparaginase by E. aerogenes. Similarly, the cell growth was inhibited at high aeration rates. This might be due to the microorganism used more substrate for its maintenance than the growth, which leads to low level of enzyme production. Aeration was varied in 24-hr production runs in 20-liter fermentors. Higher aeration rates produced greater cell production; however, total L-asparaginase production was lowered 70% [59]. Malcolm et al. 1988[60] indicated that the improved oxygen availability affected metabolites production both by increasing the rate of specific metabolites biosynthesis and by maintaining this higher rate throughout the production period. This is in agreement with our results. A 25 ml production medium in 250 ml flask gave maximum L-asparaginase production (Table 2). Also fermentation when carried out in 250 ml bottom indented flask gave maximum L-asparaginase production (Table 3).

Maladkar et al. 1993[47] reported about 6 times higher yield of L-asparaginase by the addition of L-asparagine (0.2%) to the

ISSN NO- 2230 – 7885
CODEN JPBSCT
NLM Title: J Pharm Biomed Sci.

T.A. Thaer & P. Ellaiah. J Pharm Biomed Sci. 2013, April; 29(29): 859-869.
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production medium using a mutant strain, EC-113 indicating a distinct induction of enzyme. A similar type of results was observed by[61]. They reported enhanced yield of L-asparaginase by the addition of asparagine to the production medium. The optimum level of L-asparagine was found to be 0.5 % for maximum L-asparaginase production[42]. The effect of L-asparagine as a substrate was studied with our strain A2'. The L-asparagine was incorporated into the production medium at 0.2 % level and a 50 % enhanced yield was observed (results are not shown). Our result is in good agreement with these results.

Our results revealed that optimization of medium composition allowed a significant increase in antifungal production by Streptomyces strain A2'. The highest concentration of L-asparaginase activity was produced under nutritional conditions when mannitol was used as a carbon source at 0.1 % and supplemented the medium with casein at 0.75 %. Also, initial pH of 7.0, 10 % level of inoculum, incubation period of 72 h, incubation temperature of 28°C and agitation in 250 ml bottom indented flask and medium to flask ratio of 1:10 gave maximum L-asparaginase production. L-asparagine substrate was found to be a good enzyme inducer.

The production of L-asparaginase by our isolate (A2') using all the parameters described previously was compared with that of bacterial strains B-26 and Erwinia carotovora. The B-26 is a bacterial species isolated in our laboratory and Erwinia carotovora ATCC 15713 is an L-asparaginase producing reference culture obtained from NCL, Pune, India. The results are shown in Fig. 8. The results indicated that the bacterial isolate (A2') has comparative yield of L-asparaginase with much studied Erwinia carotovora species. The literature survey indicates that Streptomycetes are less studied and showed poor yields when compared to bacteria. However, our isolate produced reasonably good amount of L-asparaginase. Strain improvement and optimization of other production parameters may enhance the L-asparaginase yield.

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ISSN NO- 2230 – 7885
CODEN JPBSCT
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T.A. Thaer & P. Ellaiah. J Pharm Biomed Sci. 2013, April; 29(29): 859-869.
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ISSN NO- 2230 – 7885
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Conflict of interest: - Author has not declared any conflict of interest.

Source of funding: - None.

*Correspondence address:
T.A. Thaer,
Department of Medical Lab Sciences,
Faculty of Allied Medical Sciences, Arab American University, P.O. Box 240, Jenin, Palestine.

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