

Summary

L-Asparaginase (L-asparagine amidohydrolase, EC. 3. 5. 1. 1) isolated from several biological sources has been shown to possess antitumor activity. Therefore, an attempt was made search for new L-asparaginase (s) from microbial source via brown pigmented streptomycetes.

The present study deals with the following main topics: -

- 1) Twenty-eight brown-pigmented streptomycetes were isolated from different fertile Egyptian soil has different asparaginolytic activities. Therefore, the L-asparaginase activity of these isolates was studied. Results showed that the highest activity was exhibited by isolate FS-39 as compared with the activity of other isolates, being 3.39IU/ml culture.
- 2) The cultural, morphological and physiological characteristics of the selected isolate (FS-39) were studied using the media and the methods of the International *Streptomyces* Project (ISP).
- 3) The streptomycetes isolate FS-39 was identified according to Kuster (1972) and Bergey's manual (1974) as *Streptomyces phaeochromogenes*.
- 4) Studying the factors affecting L-asparaginase production by *S. phaeochromogenes* revealed the following results: -
 - i- Maximal L-asparaginase production could be secured using glycerol-asparagine medium at 7th day of incubation period, under static culture condition.

ii- 0.2% L-asparagine and 2.0% glycerol (w/v) proved to be the concentrations giving maximal yield of L-asparaginase (9.1IU/ml culture).

iii- Fe^{2+} (0.0001%) is essential for the biosynthesis of L-asparaginase.

iv- 0.1% (w/v) yeast extract was the optimum concentration for maximal L-asparaginase production.

v- Studying the effect of initial pH, incubation temperature and age size of inocula on L-asparaginase production, results revealed that, the maximal yield of L-asparaginase from *S. phaeochromogenes* FS-39 could be attained under the following condition: - By growing it on glycerol-L-asparagine-yeast extract (GAY) medium which was initially adjusted to pH7.0, inoculated by 2% (v/v) of homogenized spore suspension (containing approximately 3.2×10^7 spores/ml) of 3 days old culture on starch nitrate medium and incubated at 30°C.

5) Purification of L-asparaginase produced by *Streptomyces phaeochromogenes* FS-39: - Precipitation of the enzyme from cell free culture carried out by organic solvents and ammonium sulfate at different saturation. In such precipitation it was found that the precipitation by ammonium sulfate at 50-70% saturation was more than and better purification from addition of other ammonium sulfate saturation and from addition of organic solvents (ethanol or acetone).

Trials were made for a further purification of enzyme by dissolving the precipitate in 0.05M borate buffer (pH8.5), then after, adsorption chromatography on DEAE-Cellulose column was applied. The L-asparaginase activity was detected only in fractions number (30-36). The active fractions were pooled together. Enzyme activity and protein contents were determined. Pooled fractions were subjected again to ammonium sulfate (50-70% saturation), then precipitated and dialyzed overnight against the same buffer. The dialyzed enzyme was applied to Sephadex G-200 column chromatography. The L-asparaginase activity was detected only in fractions numbers 27-31. The active fractions were pooled together and assayed for enzyme activity and protein content then after, subjected to precipitation by 50-70% saturation ammonium sulfate. The precipitate was collected and redissolved in 10ml of borate buffer at pH8.5.

The L-asparaginase of *S. phaeochromogenes* was purified 67.2 fold with overall yield of 17.7% of the original activity and specific activity 179.41IU/mg protein.

6) Characteristics of L-asparaginase (ES. 3. 5. 1. 1): -

Effect of temperature, thermal stability, pH and pH stability on the activity of L-asparaginase of *S. phaeochromogenes* FS-39 were studied. Data revealed that the optimal temperature for maximal activity was 35°C and its activity did not loose during 180min. at 35°C. Also, results revealed that the optimum pH value for the

activity of L-asparaginase was 8.5 and the enzyme was found to be stable in pH range from 8.0 to 9.0 after 180min. treatment at 30°C.

* The enzyme was shown to be stable when stored at 4°C for 7 days and for 4 days at lab. temperature (20-40°C) in borate buffer at pH8.5.

* The effect of some metal ions and some inhibitors on *S. phaeochromogenes* FS-39 L-asparaginase activity was tested. Results revealed that the activity of enzyme was slightly stimulated by addition of MgSO₄ and FeSO₄.7H₂O at concentration 1.0 and 10.0mM. ZnCl₂.7H₂O, MnSO₄.4H₂O and KMnSO₄ caused slightly inhibitory effect on enzyme activity at concentration of 0.1, 1.0 and 10.0mM. CuSO₄, AgNO₃, Iodine, L-cysteine and L-aspartic acid at concentration of 0.1mM, Iodoacetic acid at 1.0mM caused moderately inhibition on enzyme activity. While CuSO₄, HgCl₂, AgNO₃, KCN, Iodine, L-cysteine and L-aspartic acid at concentration of 1.0 and 10.0mM have highly inhibitory effect on the enzyme activity.