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Medium optimization for production of L-Glutaminase (EC 3.5.1.2) by *Streptomyces griseus* under submerged fermentation

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Abstract: L-Glutaminase is widely distributed in microorganisms including bacteria, yeast and fungi. The enzyme mainly catalyzes the hydrolysis of γ-amido bond of l-glutamine. In this report medium optimization was conducted through one -factor -at -a -time approach for the submerged production of L-Glutaminase by *Streptomyces griseus* using different additional carbon, nitrogen, amino acids, mineral salts and was treated with different concentration sodium chloride. A significant influence of medium components (g/l) Galactose 10.0,Yeast extract 10.0,L-Glutamine 10.0,Magnesium sulphate 0.5, KH_2PO_4 0.5 , K_2HPO_4 0.5, NaCl 40 on L-Glutaminase production was noted. The applied methodology was validated using this optimized media, the enzyme activity 45 IU/ml in 48h of incubation was obtained.

Keywords*:* L-Glutaminase; Production; Optimization; One factor at a time; Streptomyces griseus

1. INTRODUCTION

Microbial L-Glutaminases or Glutaminases (L-glutamine amido hydrolase EC 3.5.1.2) have found applications in several fields. L-Glutaminase activity is widely distributed in plants, animal tissues and microorganisms including bacteria, yeast and fungi. L-Glutaminase has an essential role in cellular nitrogen metabolism (1, 2, 11, 15). This enzyme gained importance in industrial and pharmaceutical sectors as an effective therapeutic agent in the treatment of HIV (16, 27) and acute lymphocytic leukaemia (22). The enzyme causes selective death of glutamine-dependent tumor cells by depriving these cells of glutamine. The use of enzymes to deprive neoplasms of essential nutrients helps in the treatment of malignancies (22) and as an analytical agent in determination of glutamine and glutamate (8, 24), as a biosensing agent in biosensor (19). L-Glutaminase enhances the flavor of fermented foods by increasing their glutamic acid content and thereby imparting a palatable taste. (6, 9) The use of L-Glutaminase as a flavour enhancing agent in Chinese foods has replaced the use of monosodium glutamate, which is considered allergic to some individuals (20). and in the production of specialty chemicals like threonine by gamma glutamyl transfer reactions (23). Its commercial importance demands the search for new and better yielding microbial strains and economically viable bioprocesses for its large-scale production (10).

Hence, Researchers are involved in the screening of microbial strains and developing different fermentation strategies to improved productivity. Bioprocess is one of the key processes which helps in enhancing the metabolite productivity under a given set of fermentation environment (12, 13). Improvement in microbial metabolite production is generally attempted by manipulating the nutritional and incubational parameters of the organism. Combinatorial interactions of medium components with the cell metabolism towards the production of the desired compound are plentiful, and the optimum processes may be developed using an effective experimental design procedure.

To our knowledge reports on the production of L-Glutaminase from *Streptomyces griseus* is scanty. It's an aerobic gram positive filamentous bacteria .In the present investigation, one-factor-at-a-time approach was used to select the best combination of carbon, nitrogen, amino acids, sodium chloride and minerals salts sources and validated the impact of mixed sources on production by *Streptomyces griseus* under submerged fermentation.

2. MATERIALS AND METHODS

2.1 Medium Components

Nutrient broth, L-glutamine, Nessler's reagent and other media components and chemicals were procured from Hi-Media Limited, Mumbai, India. For optical density

measurements, the absorbance was read using UV/Vis Bio Spectrophotometer (EliCo Pvt. Ltd., India).

2.2 Microorganism and Culture maintenance

Streptomyces griseus NCIM 2622 procured from NCIM, National Chemical Laboratory, India, was used in the study. The culture was maintained on Nutrient agar medium slants. Inoculated slants were grown in an incubator at 33 ^{o}C for 4 days. After that the slants were stored at 4 ^{o}C in a refrigerator for short-term preservation and sub-cultured every 15 days in the above-mentioned media.

2.3 Inoculum preparation

Inoculum was prepared in 250 ml Erlenmeyer flasks containing 100 ml of Nutrient broth liquid medium (pH 7.0). Prepared medium was autoclaved at 121 ^{o}C (15 lb) for 20 min and then inoculated with *Streptomyces griseus* raised from Nutrient agar slants. The inoculated flasks were kept on a shaker at 150 rpm for 48h, and used as the inoculum.

2.4 Identification of medium components

Initially optimization of media components required for maximum L-Glutaminase production by *Streptomyces griseus* was evaluated in 100ml of 250 ml Erlenmeyer flasks at 33 ^{o}C for 48 hr at 150 rpm by adding 0.002% of inoculum. The L-Glutaminase production on nutrient broth was used as a control .Subsequently the medium component studied included the effect of different additional carbon sources (Malt extract, D-glucose, Sucrose, Starch soluble, Tri sodium citrate, Cellobiose Cellulose, D-mannitol, Lactose, Galactose, D-fructose, Maltose) at10 g/l, effect of additional nitrogen sources (Peptone, Sodium sulphite, Yeast extract , Urea, Tryptone, Gelatin, Sodium nitrate) at 10 g/l, effect of additional amino acids (L–glutamic acid, Glycine, L –ascorbic acid, L –glutamine, Cysteine, Alanine) at 10 g/l,effect of additional minerals(Zinc sulphate, Mercuric sulphate, Manganous sulphate, Copper sulphate, Ferrous sulphate, Magnesium sulphate , Potassium di hydrogen phosphate, Di potassium hydrogen phosphate, Calcium chloride) at 0.5g/l . After identifying the nutrients improving L-Glutaminase production by 'one factor-at-a-time' approach, the four most important nutrients, viz. Galactose, yeast extract, glutamine and Magnesium sulphate were selected as a medium components and finally the effect of sodium chloride concentration (10–50g/l) on above said medium was studied. All the fermentation experiments were carried out in triplicate. The optimum media was identified as (g/l) Galactose10.0, yeast extract 10.0, glutamine 10.0, Magnesium sulphate 0.5, Potassium di hydrogen phosphate 0.5, Di Potassium hydrogen phosphate 0.5 and Sodium chloride 40.0, on L-Glutaminase production was observed at 48 h.

2.5 Analytical determinations

At appropriate time intervals the fermentation broths were harvested for the L-Glutaminase enzyme. The broth was centrifuged at 10000 rpm for 20 min at 4 ^{o}C in a refrigerated centrifuge and the supernatant obtained was used for further enzyme assay procedures.

2.6 Determination of Enzyme activity

L-Glutaminase was assayed according to Imada et al (7). The reaction mixture, containing 0.5ml of an enzyme preparation ,0.5 ml of L-glutamine(0.04 M), 0.5 ml of phosphate buffer 0.1 M (pH 8.0), and 0.5 ml of distilled water to a total volume of 2ml solution was incubated at 37°C for 30 min. The reaction was stopped by addition of 0.5 ml of 1.5 M Tri chloro acetic acid. Then to 3.7 ml of distilled water, 0.1 ml of the above mixture and 0.2 ml of Nessler's reagent were added and colour developed was read after keeping the mixture at 20°C for 20 min at 450 nm in a spectrophotometer .Enzyme and substrate blanks were used as controls. One unit of L-Glutaminase activity was defined as the amount of enzyme that liberated 1µmol of ammonia per1min under optimal assay conditions. Assays were done in triplicate and the mean enzyme activity was expressed as International unit per ml (IU/ml).

3. RESULTS AND DISCUSSION

3.1 Identification of medium components

L-Glutaminase production by a *Streptomyces griseus* under submerged fermentation condition was observed during the course of study and the observation led to an investigation of the potential of L-Glutaminase synthesis towards developing an ideal bioprocess for industrial production of this enzyme. Hence initially the various nutrients and process parameters, which influence L-Glutaminase production by *Streptomyces griseus* under submerged fermentation conditions, were optimized.

3.2 Effect of additional carbon sources

Carbon source represents the energy source that will be available for growth of the microorganism. Carbohydrates and related compounds are superior carbon sources for many genera of microbes (18). However, in some cases, addition of a small amount of external carbon may lead to an increase in

enzyme production. Fig.1 showed the effect of additional carbon source for yield of L-Glutaminase from *Streptomyces griseus* was variably changed, when the carbon source changed. In this work, we found yield of L-Glutaminase was high as 26.3 IU/ml by utilized the Galactose as the carbon source.

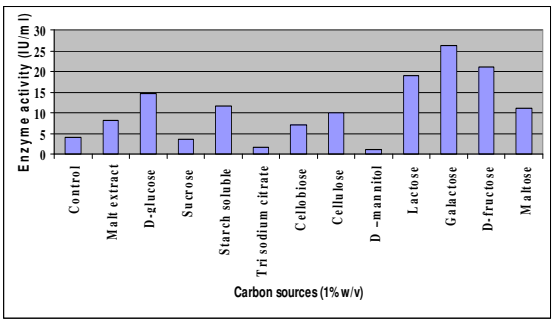

Figure 1: Yield of L-Glutaminase in different carbon source.

3.3 Effect of additional Nitrogen sources

Effect of different nitrogen sources (Fig.2) showed that the maximum yield was obtained as 17.5 IU/ml in presence of yeast extract, because the yeast extract serves as complex Nitrogen source for the metabolic activity. Universal ingredient yeast extract was normally added to media for routine growth and amino acid supplementation was not required in complex media containing yeast extract.

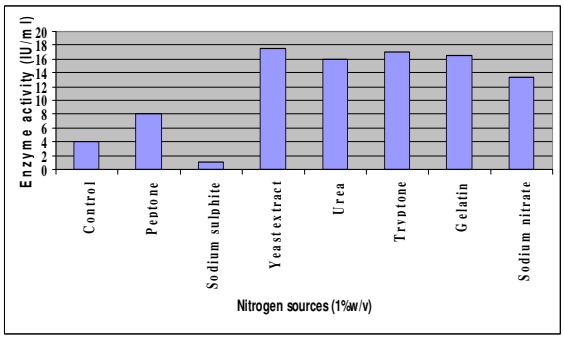

Figure 2: Yield of L-Glutaminase in different Nitrogen source.

3.4 Effect of additional amino acids sources

Amino acids were common growth factor required for the synthesis of enzyme as major nitrogen source (4); hence the yield of L-Glutaminase was varied, when the amino acid was changed. Even though each and every amino acid was interchanged by other amino acids, the L-Glutaminase yield was varied according to the nature of amino acids (Fig.3). Yield of L- Glutaminase from the *Streptomyces griseus* was high as 32.7 IU/ml in L-glutamine. Since L-glutamine is the substrate of L-Glutaminase, the addition to fermentation medium might stimulate enzyme production. It also serves as source of energy and carbon.

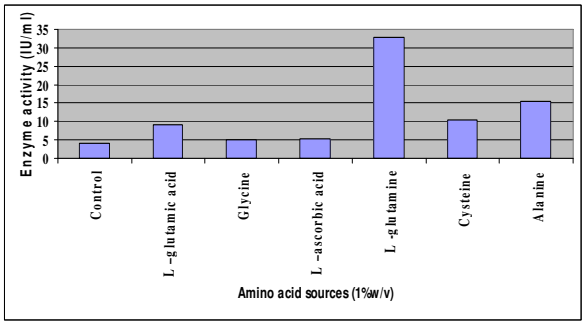

Figure 3: Yield of L-Glutaminase in different Amino acid sources.

3.5 Effect of additional mineral salt sources

All the living organisms need some inorganic nutrient for their growth, that do not usually contain the element carbon and when it dissolve in water they separate into ions.L-Glutaminase yield obtained from *Streptomyces griseus* in the presence of different mineral salts (Fig.4) showed that the maximum yield was 35 IU/ml in the presence of Magnesium sulphate, $KH_2PO_4$and K_2HPO_4 which is supported both enzyme production and the bacterial growth (5, 14, 21, 25, 26).

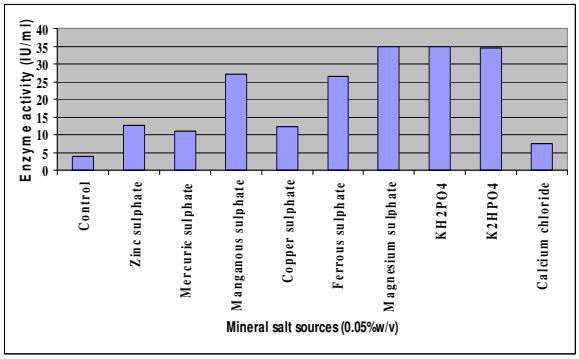

Figure 4: Yield of L-Glutaminase in different Mineral salt sources.

3.6 Effect of additional sodium chloride

Yield of L-Glutaminase was increased, when increased the NaCl concentration up to 4% as maximum as 45 IU/ml and it was low in 1%, 2%and 3% of NaCl concentrations (Fig.5). Yield was suddenly decreased, when the concentration was increased above the 4%.Hence, 4% of NaCl concentration was the optimum for the production of L-Glutaminase from *Streptomyces griseus*. The bacteria didn't

produce more L-Glutaminase without the NaCl because the *Streptomyces griseus* were halophilic, the bacteria were unable or try to grow in the low NaCl concentration, hence there was very low L-Glutaminase production and also the high concentration of NaCl was also affect the growth of bacteria.

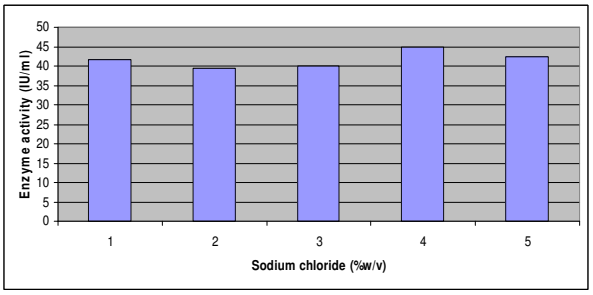

Figure 5: Yield of L-Glutaminase in different concentration of Sodium chloride.

Optimum levels of these significant sources and the effect of their interactions on L-Glutaminase productions were determined by the one –factor- at -a time. The optimized medium components (g/l) Galactose 10.0, Yeast extract10.0, L-Glutamine10.0, Magnesium sulphate 0.5, KH_2PO_4 0.5, K_2HPO_4 0.5, NaCl 40.0 on L-Glutaminase production was noted, which gave the maximum enzyme yield of 45 IU/ml.

4. CONCLUSION

In this work medium components for higher L-Glutaminase production from *Streptomyces griseus* were optimized by one- factor- at -a time approach. Using one factor at a time approach (g/l)Galactose 10.0,Yeast extract 10.0,L-Glutamine 10.0,Magnesium sulphate 0.5, KH_2PO_4 0.5 , K_2HPO_4 0.5 NaCl 40.0 were found to be the most significant variables, which significantly enhanced L-Glutaminase production. Using these optimized conditions, the produced enzyme activity of L-Glutaminase reaches 45 IU/ml.

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