Mintage Journal of Pharmaceutical & Medical Sciences

Vol 7, Issue 1, 2018 ISSN: 2320-3315

MINTAGE JOURNALS

Research Article

PRODUCTION OF L-ARGINASE UNDER SSF AND ITS OPTIMIZATION

RAHAMAT UNISSA*, KOMATI BHANU REKHA, DAGGUPATI AMANI, SOWMYA KONAKANCHI, SARANYA ARUN KUMAR

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Malla Reddy College Of Pharmacy, Maisammaguda, Dhulapally, Secunderabad, Osmania University, Telangana, India. Email: srunissa@gmail.com, syedaunissa37@gmail.com

Received - 01.12.2017; Reviewed and accepted - 22.12.2017

ABSTRACT

Objective: The present study deals with the fermentative production of l-arginase under solid state fermentation technique .Various nutritional as well as physical parameters were altered in order to maximize the yield. **Method:** L-Arginase has received significant attention in recent years owing to its potential applications in medicine as an anticancer agent, as an efficient diagnostic agent and as a biosensor. Production of the enzyme was carried out by using various solid substrates such as wheat bran, tea dust, rice bran, ragi, maize bran, black gram, bread, red gram as solid substrates. Various factors such as incubation periods (24-168 hrs), p H (5-10), inoculum size (2.5-15%), incubation temperature (20-450C), l-arginine concentration (0.5-3%), NaCl concentration (1-5%) etc. were altered and their effect on the production rate was noticed. **Results:** The maximum yield (66.08 IU/ml) of l-arginase by Idiomarina sediminium was obtained using tea dust as a solid substrate, initial pH 9.0, supplemented with maltose (1.0%), casein (1.0%), inoculated with 10% of inoculum and incubated at 37°C for 120 h. **Conclusion:** Solid state fermentation method can be adopted for large scale production of the enzyme for the therapeutic use in the future.

Keywords: L-Arginase, Idiomarinasediminum; H1695; JF346667, tea dust, Solid state fermentation.

INTRODUCTION

L-Arginase (EC 3.5.3.1) is a manganese containing enzyme that catalyzes the irreversible hydrolysis of l-arginine to l-ornithine and urea [1]. This enzyme is classified under ureohydroloase family.It is wide spread in the biological world, ranging from bacteria to human beings have the enzyme[2].Interest on l-arginasesstarted with the exploration of their anti-tumour properties and since then, numerous efforts have been made to conduct the detailed studies on the microbial arginases with an aim to develop it in the form of a chemotherapeutic drug.

Cancer, mainly leukemia, is a global problem and in spite of several efforts made in the past, search for more efficient drugs is still being continued globally [3]. Interest in ureohydroloasessuch as l-arginases started with the discovery of their antitumor properties. Most of the tumorsare dependent upon l-arginine for their growth and development and they are auxotrophic in nature. They totally depend upon the exogenous supply of the amino acid for their growth. L-Arginase converts the l-arginine available into l-ornithine and urea there by depleting the levels of the amino acid. As these tumour cells divide abnormally, their requirement for the amino acid is also high. One way to arrests the growth of these tumours cells is to stop dietary intake of l-arginine and to deplete the already present amino acid by the action of the enzymes such as l-arginase [4]. Arginine deprivation has been proven to be a promising treatment option for arginine auxotrophic cancers, such as melanoma, lung cancer, renal cell carcinomas and hepatocellular carcinomas [5, 6, 7,8].

L-Arginases are widely distributed in all kingdoms of life. They are also present in the liver of the human beings. But it is often associated with huge cost and difficulty in the separation. Microorganisms are considered as potential sources of these enzymes. Microbial anticancer enzymes are of appreciable interest in view of their specific activity and stability at biological pH.

In our preliminary studies we have isolated the potential enzyme producer from the marine isolates (Idiomarina sp.)[9].Same microorganism was employed for the production of the enzyme in the present study. The present work deals with the production of l-arginase by solid state fermentation technique.

In recent years, solid state fermentation (SSF) has emerged as a promising technology for the development of several bioprocesses which include the production of industrial enzymes on a large scale.

Solid state fermentation (SSF), that consisted growing of micro-organisms on moist solid supports in the absence of free-water, is a promising technique for the large scale production of useful microbial enzymes, antibiotics, organic acids, food flavours, alkaloids and other bioactive secondary metabolites [10]. The success of SSF processes mainly depend on the type of the strain and the substrate used. Marine microorganisms are reported to be highly suitable for use in SSF because of their salt tolerance, ability to produce novel metabolites and the capacity to adsorb onto the solid particles [11].

This paper deals with the production of l-arginase produced by marine bacterium, Idiomarina sediminum; H1695; JF346667 using tea dust as a novel substrate

MATERIALS AND METHODS

Chemicals

Chemicals used in the present study for the preparation of the media were obtained from Hi-Media laboratories. Remaining chemicals were purchased from Sigma Aldrich, Bengaluru, India.

Bacterial strain: Idiomarina sediminium H1695 was employed for the production of the enzyme. It was isolated from the water samples procured from the coastal areas of Andhra Pradesh. It was tested for the enzyme production and identified by sequencing method [9].

Inoculum: Fresh cultures were employed for the preparation of the inoculum. Sufficient amount of the sterile water was incorporated into the NA slants of the microorganism, mixed well and poured into sterile nutrient broth medium. It was then incubated for 24hrs at 37^0 C in an incubator. 10% of suspension was used as an inoculum.

Preparation of solid substrate: Various solid substrates like wheat bran, tea dust, rice bran, ragi, maize bran, black gram, bread, red gram were employed for the enzyme production and their effect on the enzyme production was studied.5Grams of these substrates were taken in separately in 100ml of conical flask and dampened with 5ml of sea water based mineral medium. It was then sterilized by moist heat at 121^0C for 15min.2ml of the inoculum was incorporated into the flasks and incubated at 37^0 C for 48 hrs.

Inoculation and incubation: The flasks were cooled and inoculated with 2ml of inoculum and incubated in slanting position in an incubator at 35^0 C for 24-28hrs.

Extraction and recovery of enzyme

The protein was separated using simple contact method described by Prakashamet al,2006[12].The enzyme from the fermentable substrate was extracted by using 0.1 M phosphate buffer (p H 8).40ml of buffer was added into the flask and was mixed for 30 min at 150rpm.The slurry formed was centrifuged at 10,000 rpm for 10min in a cooling centrifuge maintained at 4^{0}C.The supernatant thus obtained was used as crude enzyme.

Quantitative estimation of urea

Enzyme activity was determined in terms of the rate of hydrolysis of l-arginine to l-ornithine and urea. The quantity of the urea formed was determined by the method followed by Archibald[13]. Standard graph for urea estimation was plotted by using different concentrations of urea.

Quantitative estimation of protein

The amounts of the proteins present in the crude extract was determined by Lowry's method (1951)[14].Standard curve for protein estimation was made by using BSA.

Production and optimization

Optimization of the various process parameters required to maximize l-arginase production by Idiomarina sediminium was performed. Solid state includes use of different solid substrates(Wheat bran, tea dust, rice bran, ragi, maize bran, black gram, bread, red gram), Incubation periods (24-168 hrs), p H (5-10), inoculum size (2.5-15%), incubation temperature (20-45^{0}C), l-arginine concentration (0.5-3%), NaCl concentration (1-5%),carbon sources (glucose, sucrose, mannitol, maltose, glycerol, starch, mannose), nitrogen sources (yeast extract, beef extract, casein, gelatin, NH_4NO_3, NH_4Cl, peptone, tryptone, soya bean.

The procedure employed for maximization of various parameters keeping others constant and to incorporate it at the optimized level in the next experiment while optimizing next parameter.

RESULTS AND DISCUSSIONS

Solid state fermentation offers several advantages over other conventional fermentations, such as submerged fermentation etc. Solid state fermentation was conducted for the production of l-arginase using different agroresidues including wheat bran, tea dust, rice bran, ragi, maize bran, black gram, bread, red gram as solid substrates. Tea dust was the best substrate for induction of l-arginase.

The major advantages of SSF includes higher product yields, lower capital and recurring expenditure, lower waste water output/less water need, reduced energy requirement, absence of foam formation, simplicity, high reproducibility, simpler fermentation media, lesser fermentation space, absence of rigorous control of fermentation parameters, economical to use even in smaller scales, easier control of contamination, applicability of using fermented solids directly, storage of dried fermented matter, lower cost of downstream processing. Problems generally linked with SSF are heat build-up, bacterial contamination, scale-up, biomass growth assessment and control of process parameters.

SSF yields a high product concentration and has a relative low energy requirement. Because of mere absence of water, small fermentors are required for SSF and therefore less strain is needed for purification process. Wild type cultures of bacteria and fungi tend to perform better in SSF conditions than do genetically manipulated microorganisms, reducing energy and cost requirement further. Microorganisms make use of various substrates as a source of nutrient for their growth and metabolic activities. In SSF, microorganisms secrete necessary enzymes for the breakdown of the available substrates molecules in order to meet their nutritional requirements.

Effect of different solid substrates

The solid substrates employed in the solid state fermentation are generally insoluble in water and play a dualrole of supply of nutrients to the microbial culture growing and anchorage for the growing cells. The critical factor involved in SSF is the choice of suitable substrates [15]. The testing of numerous substrates for optimal l-arginase production clearly shows the variation of the enzyme productivity for various substrates used. The optimal enzyme productivity was noticed for tea dust followed by wheat bran. Tea dust is an ideal carrier and supporter because of its porosity and its availability. Our results correlate with those of K. Nathiyaet al. It was reported that coconut oil cake was found to be a potential raw material for the production of α-amylase [16].

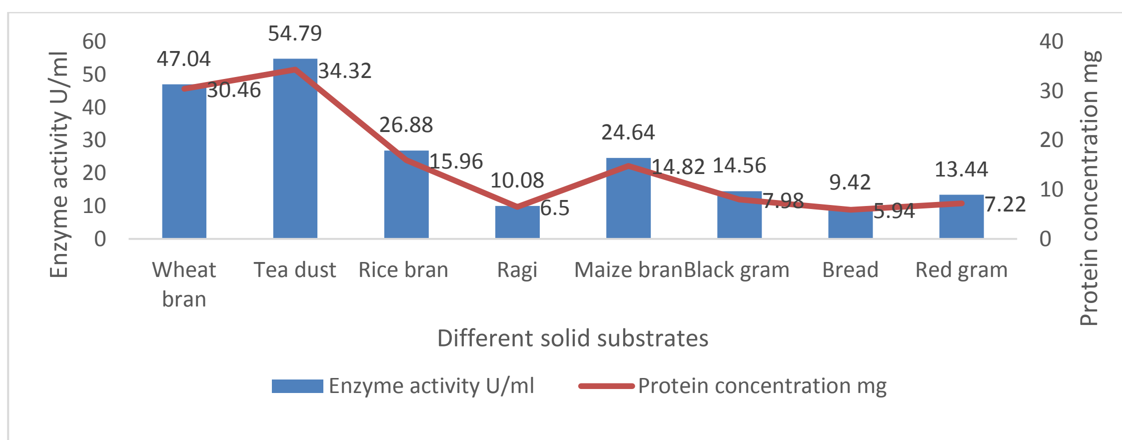

Fig. 1: Effect of different solid substrates on l-arginase production.

Effect of incubation periods

Incubation temperature also showed marked influence on the rate of l-arginase production by marine organism. Maximum production of the enzyme occurred after 120hr of incubation. L-Arginase production showed progressive increase from 24 hrs reaching a maximum at 120hrs and further incubation lowered the production considerably. In case of production of l-glutaminases from Aspergillusoryzae optimal incubation period was found to be 48 hrs. Same type of results were seen in the studies carried by P. Kashyapet al using Z. rouxii[17].

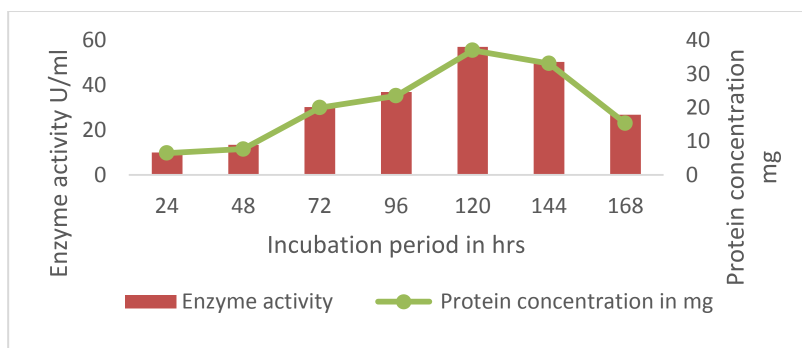

Fig. 2: Effect of incubation periods.

Effect of initial pH

The pH of the fermentation medium is reported to effect the growth of bacterial strain as well as the product formation. Increase in the pH increased the production of the enzyme. Optimal enzyme production took place at pH 9. Further increase, decreased the enzyme synthesis. Results also suggest this marine bacterium is alkalophilic in nature. These results were in coincidence with that reported by Sabuet al. [18].

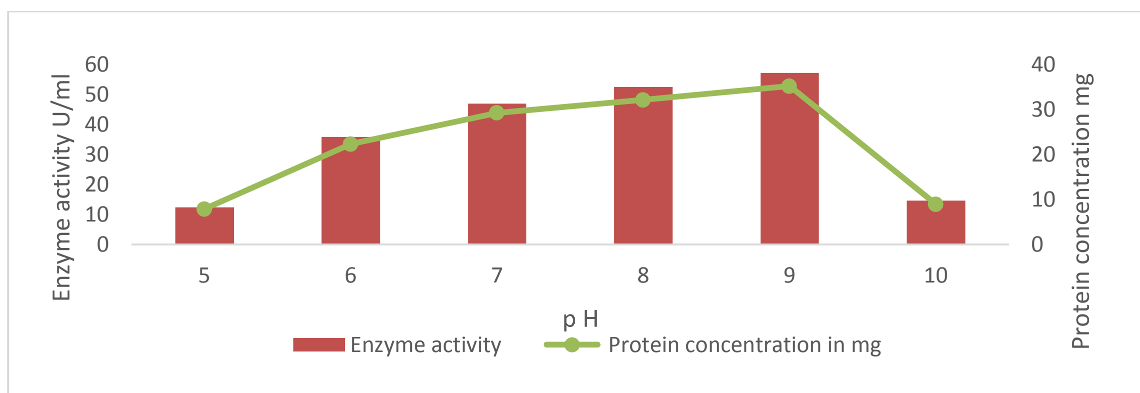

Fig. 3: Effect of pH on L-Arginase production

Effect of inoculum size

The initial inoculum volume controls growth rate as well as diverse metabolic functions resulting in overall biomass and extracellular product formation. To check out the same, experiments were programmed with increasing inoculums concentration from 2.5 to 15 %. L-Arginase synthesis increased by increasing concentrations of inoculum. The maximum l-arginase yield was achieved at 10% of inoculum and further increase in the inoculum did not produce any significant impact on the enzyme yield which might be due to the nutrient limitations.

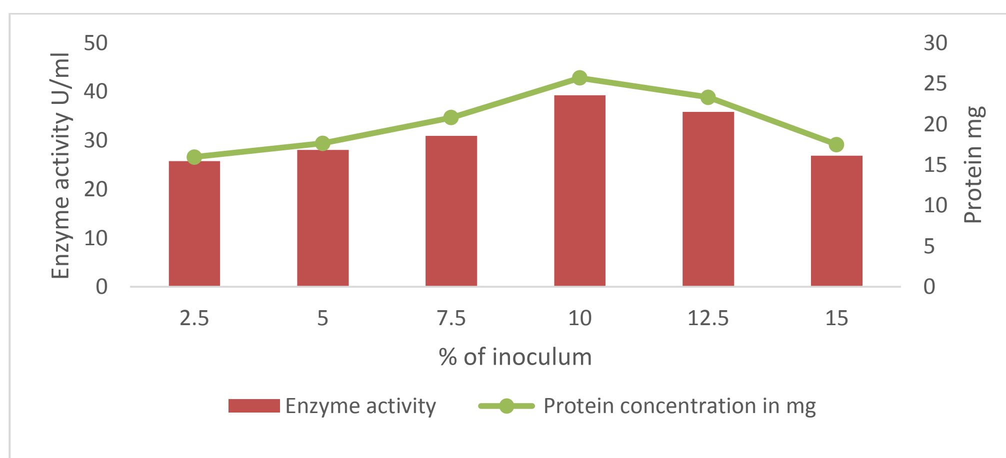

Fig. 4: Effect of inoculum size

Effect of incubation temperature

Incubation temperature plays essential role in the enzyme production. For higher production rates, the medium was exposed to different temperatures. L-Arginase production increased with the increase in the temperature. The optimal temperature at which the maximum production of the enzyme occurred was found to be 37^0 C. Enzyme production declined sharply beyond this temperature. Although, the physiological modifications instigated by high temperatures during enzyme production are not totally

understood, it has been recommended that at high temperatures, microorganisms may produce only diminished levels of proteins necessary for growth and other physiological processes [19]. Same type of results was reported for glutaminase from Trichodermakoningii which produced 15.59 U/gds at 33°C [20]. With prolonged incubation, enzyme activity declined sharply indicating that the end-point of fermentation should be precisely controlled because the synthesized enzyme could be broken down by non-specific proteases secreted by the bacteria.

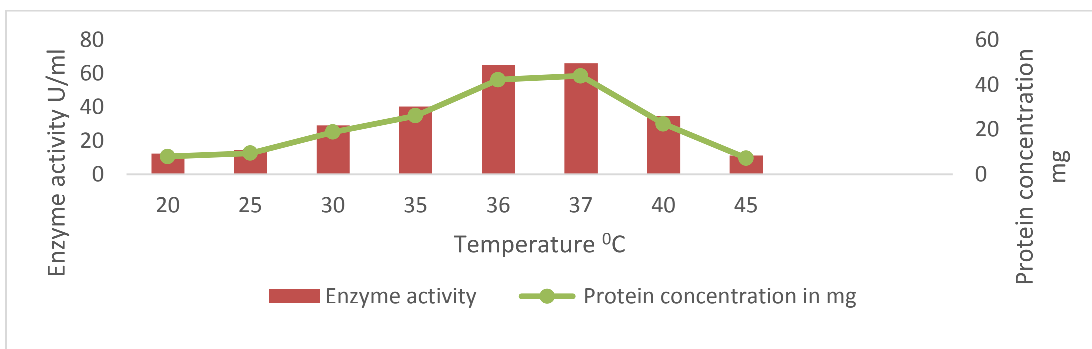

Fig. 5: Effect of incubation temperature

Effect of inducers (Amino acids)

Among the different amino acids tested, l-arginine was observed to enhance el-arginase production. L-Arginase production occurred even in the absence of l-arginine as well as any additional amino acid in the seawater medium. This particular study recommends that Idiomarinas sp. could produce extracellular l-arginase even in the absence of an enzyme inducer, when seawater was employed as a medium. An elaborate investigation on the molecular mechanism associated in the role of seawater contents in the biosynthesis of l-arginase would yield information on the biology of these organisms in natural environment alongside designing an economically viable fermentation media.

Amount of the inducer used has significant effect on the enzyme production. Highest enzyme production occurred at 2% concentration.

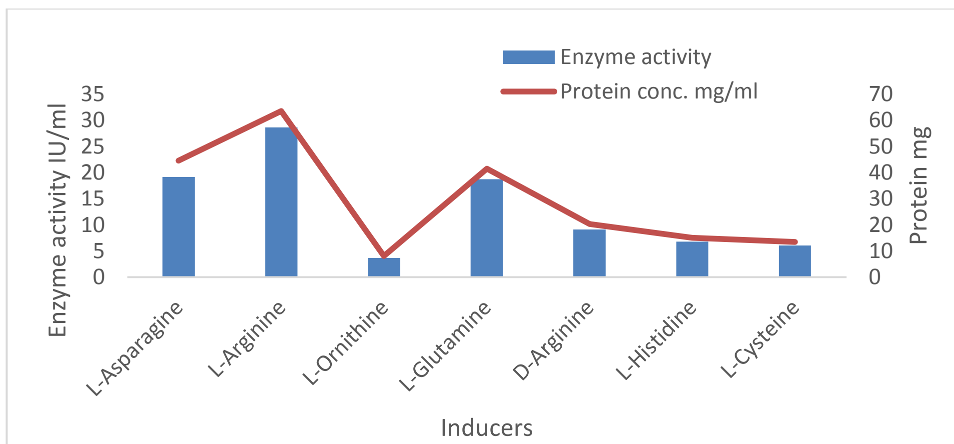

Figure 6: Effect of inducers

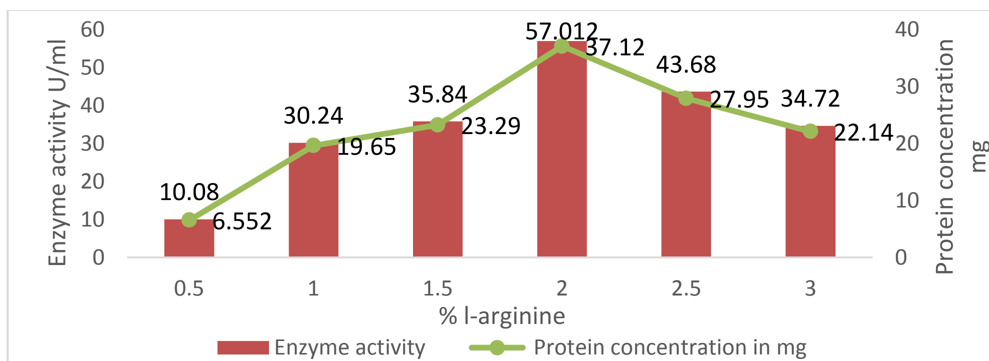

Fig. 7: Effect of inducers

Effect of sodium chloride

The NaCl requirement for the enzyme production is shown in the figure 8 given below. It was noticed that 4% of the NaCl was optimal for the enzyme production. Further increase in the NaCl concentration did not improve the yield.

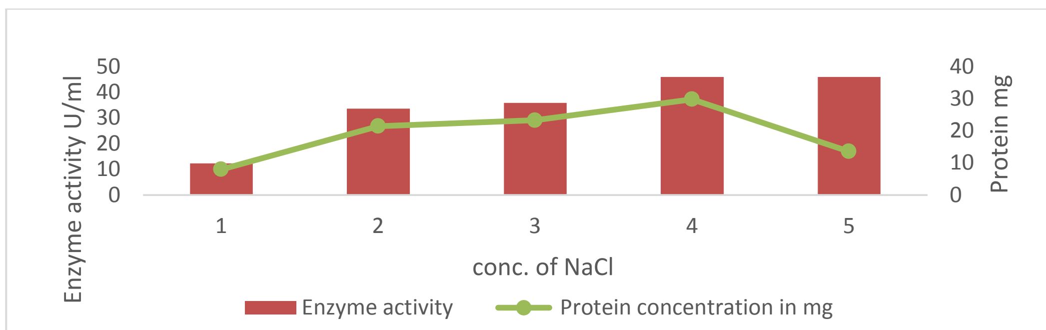

Figure 8: Effect of sodium chloride

Effect of carbon sources

Out of various carbon sources, maltose was found to be optimal carbon source. The effect of various carbon sources on the l-arginase production is given under the figure 9 given below. These results were identical to those recorded by production by Vibrio costicola by Prabhu and Chandrasekaran [21].

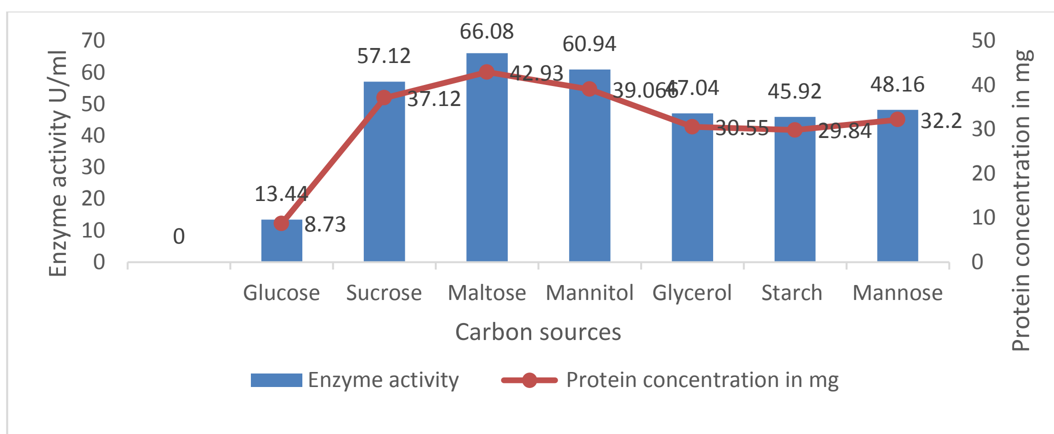

Fig. 9: Effect of carbon sources

Effect of nitrogen sources

Nitrogen source can be an important limiting factor in the microbial production of enzymes. The impact of various nitrogen sources on the l-arginase production is shown in the figure 10 given below. Out of various nitrogen sources casein was found to be optimal for the enzyme production. From the result, it was also noticed that none of the inorganic nitrogen sources properly promoted the enzyme synthesis as compared to organic nitrogen source. In case of production of l-argininінedeiminase using marine Vibrio alginolyticus, soya bean meal was found to an optimal nitrogen source [22].

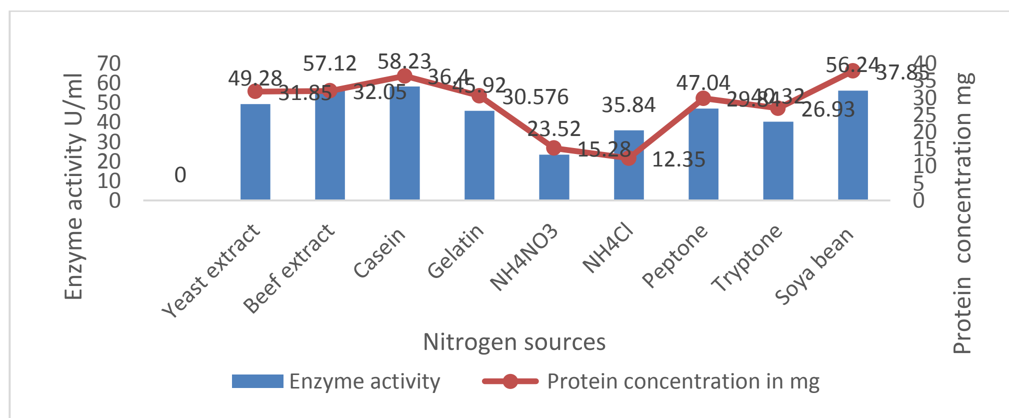

Fig. 10: Effect of different nitrogen sources.

CONCLUSION

The strain obtained from marine source was a non-pathogenic strain. The present study shows that marine strain, IdiomarinasediminumH1695; JF346667can produce relatively good yield of l-arginase from tea dust as a substrate which is easily available and economical. Marked improvement in the yield of l-arginase was noted when the basal medium was enriched with different carbon and nitrogen sources. It is also noted that the incorporation of l- arginine to the nutrient media resulted in the marked advancement in the yield ofl-arginase. This study indicates that tea dust from agro industrial residues is a good substrate for the cultivation of Idiomarina sediminum; H1695, to produce l-arginase by SSF. This process can be exploited for the large scale production of the enzyme, which has wider application in the field of food and pharmaceutical industries.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' DECLARATION

The authors hereby declare that the work presented in this paper is original and that any liability for claims relating to the content of this article will be borne by them.

ACKNOWLEDGEMENT

Authors are thankful to the authorities of Malla Reddy College of Pharmacy, Telangana, India for providing the facilities to carry out this work.

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