Isolation, screening and optimization of L-asparginase producer *Bacillus* sp. from agricultural soil

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Abstract: In recent years, microbial L-asparaginases have drawn particular attention because of their potential antineoplastic properties and significant application in food industries. In present study, total 67 bacterial isolates were isolated and screened for L-asparginase production from agricultural soil on a Modified M9 agar medium with phenol red as an indicator. Among 67, 19 isolates showed L-asparginase production ability. Among which one isolates *Bacillus* sp. showed highest production and was optimized for various condition for better results. The result suggests that optimum pH, temperature, source of carbon and source of nitrogen for L- asparginase production by *Bacillus* sp. was 8, 37^0C, 0.8% glucose and 1.5% yeast extract, respectively.

Keyword: *Bacillus* sp., enzymes, L- asparginase, optimization.

Introduction:

L-asparagine is an essential amino acid used for nutritional requirement of both normal cells and cancer cells. L-asparaginase is the enzyme that cleaves L-asparagine into aspartic acid and ammonia. Since several types of tumour cells require L-asparagine for protein synthesis they are deprived of an essential growth factor in the presence of L asparginase (Gosh et al., 2011). This enzyme is widely used in the treatment of acute lymphatic leukaemia mainly in children's. Lymphatic tumour cells require huge amount of L-asparagine for malignant growth (Bansal et al., 2010).

L-asparaginase was produced by wide range of organisms such as bacteria, fungi, actinomycetes, algae and plants However, microbes are good source as compared with others, because they can easily cultured, extraction and purification as well as the methods of this process from them is also convenient (Gupta et al., 2009; Kamble and Khade, 2012). Hence, the present study focuses on the isolation, screening and optimization of L-asparginase producer bacteria from agricultural soil.

Sample collection:

Four soil samples were collected from different agricultural field at a depth of 30 to 40 cm in and around Loni region, District Ahemednagar. The samples were collected into the sterile polythene bags and carried to the laboratory for further microbial analysis.

Isolation, identification and screening of bacteria:

The 1 g of soil sample samples was serially diluted with sterile distilled water. The last dilution were inoculated on nutrient agar medium by spread plate method and incubated at 37^0C for 24 hr. After incubation, well isolated colonies were purified and maintained on nutrient agar slant and stored at 4^0C. All the isolates from nutrient agar were inoculated on grown on Modified M9 medium (composition (g/l): KH_2PO_4 2.0, L-asparagine 6.0, $MgSO_4.7H_2O$ 1.0, $CaCl_2.2H_2O$ 1.0, glucose 3.0, and agar 20.0) supplemented with phenol red as indicator for screening the L-asparaginase producer (Prakasham et al., 2010) and the plates were incubated at 37^0C for 24 hrs. Those colonies that displayed pink red colour were consider as L-asparaginase producer and were identified on the basis of morphological and biochemical tests.

Enzyme production

L-asparaginase production by the isolate was carried out by submerged fermentation. The sterilised production media (composition (g/l): KH_2PO_4 2.0, L-asparagine 6.0, $MgSO_4.7H_2O$ 1.0, $CaCl_2.2H_2O$ 1.0 and glucose 3.0) was inoculated with a loop-full of log phase bacterial culture and was incubated in a rotary shaker at 37^0C at 200 rpm for 48 hrs. At the end of incubation, culture filtrates were obtained by centrifugation at 8000 rpm for 15 min and the supernatant was then used as crude extract for L-asparaginase activity.

Optimization of L-asparaginase production:

The effect of different parameters on the L-asparaginase production by the isolate was optimized for various conditions.

Effect of pH:

The effect of pH on L-asparaginase production was studied by growing the isolates in sterile production medium of different pH (5, 6, 7, 8, 9 and 10) and incubated at 37^0C for 48 hrs and assayed for enzyme activity. The pH was maintained by using phosphate buffer.

Effect of Incubation Temperature:

The effect of incubation temperature on L-asparaginase production was studied by growing the bacterial isolates in sterile production medium were incubated at different temperatures (25^0C - 45^0C) for 48 hrs and assayed for enzyme activity.

Effect of Carbon Source Concentrations

Glucose was used as carbon source at different concentrations (0.2 to 1.2%) were added into the production medium separately and incubated at 37^0C for 48 hrs and assayed for asparaginase activity.

Effect of Nitrogen Source Concentrations

Yeast extract was used as source of nitrogen at different concentration were added into the production medium separately and were incubated at 37°C for 48 hrs and assayed for enzymatic activity.

Assay of L-asparaginase activity:

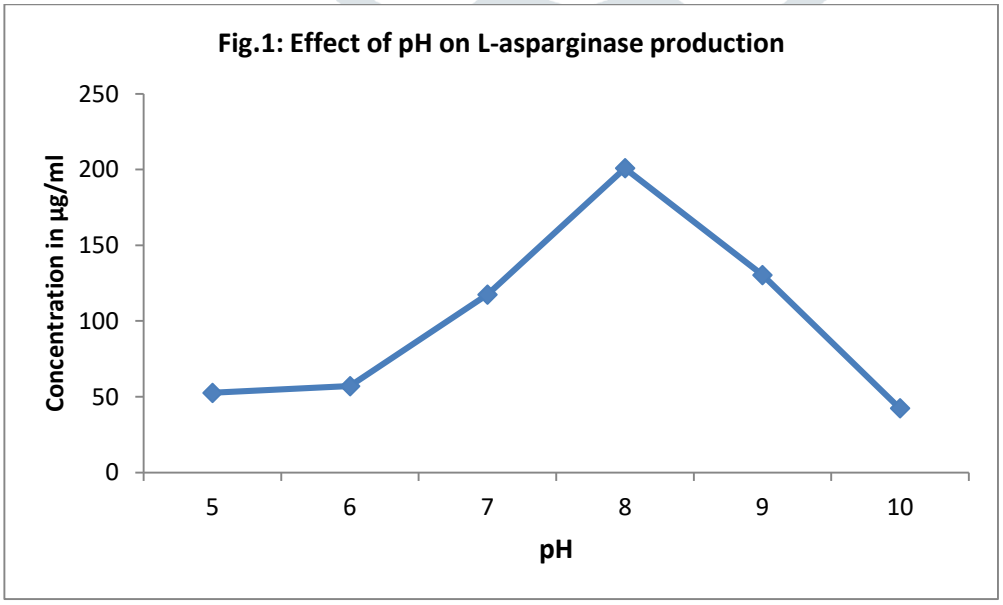
L-asparaginase activity was determined in culture filtrates by quantifying the ammonia formation using nessler's reagent. The enzymatic reaction mixture contains 0.5 ml of 0.04 M L-asparagine substrate, 0.5 ml of 0.05 M Tris-HCl buffer (pH 8.6) and 0.5 ml of crude enzyme. The enzyme substrate mixtures

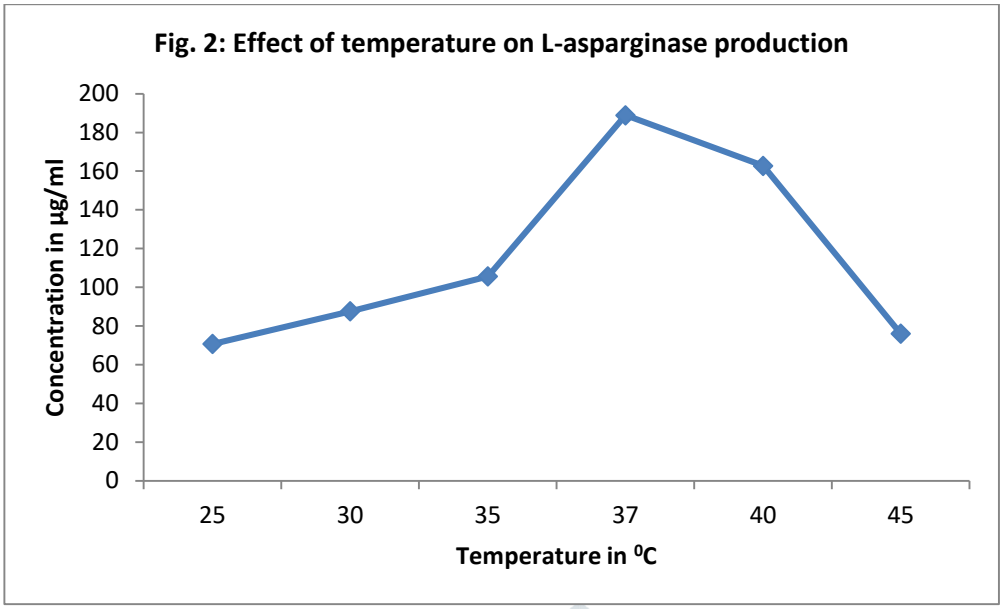
were incubated at 37°C for 30 min. After incubation period the enzyme activity was stopped by the addition of 0.5 ml of 1.5 M trichloroacetic acid. The liberated ammonia was coupled using nessler's reagent incubated at 20°C for 15 min for development of colour that was measured at 450 nm using UV-visible spectrophotometer. The liberated ammonia was determined by inference from the standard curve of ammonium sulphate.

Results and discussion:

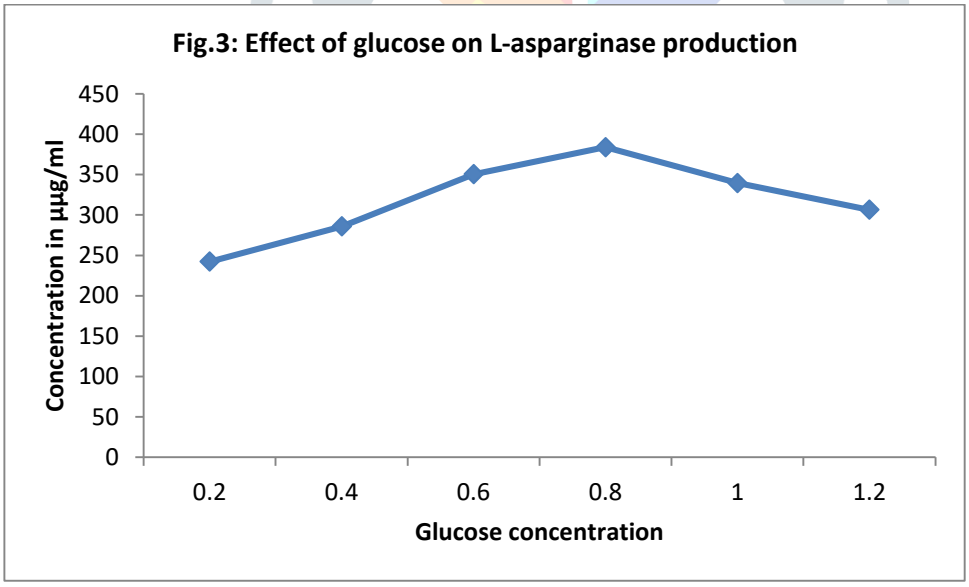
In present study, total 67 bacterial isolates were isolated from agricultural soil on nutrient agar. Among them, 19 bacterial isolates showed L-asparginase production ability on a modified M9 agar medium with phenol red as an indicator. Out of 19, one isolates showed highest production of L-asparginase which was identified as *Bacillus* sp. on the basis of morphological and biochemical tests. Further, *Bacillus* sp. was optimized for various conditions for better results. Similar studies were reported earlier (Kamble et al., 2012; Lalitha Devi and Ramanjaneyulu, 2016).

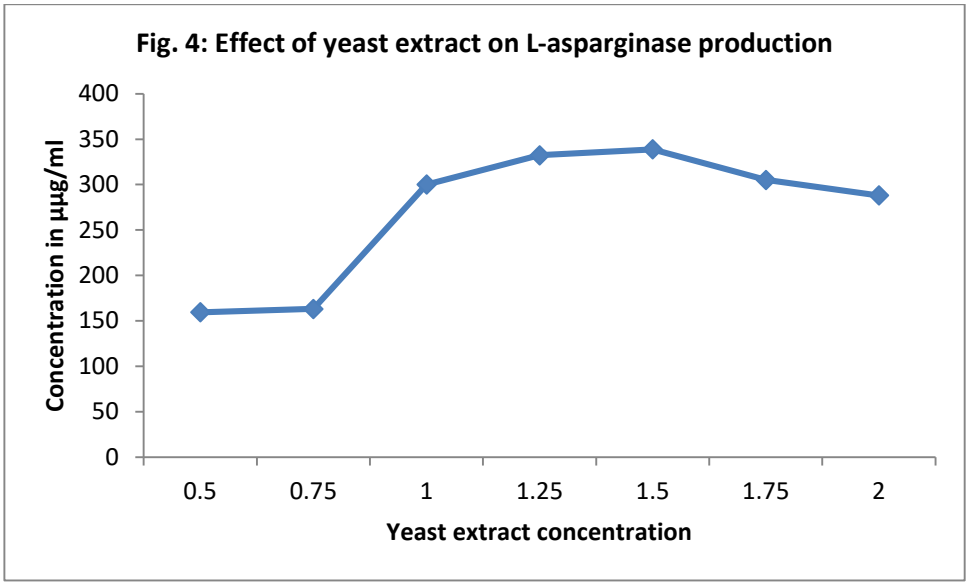
The extracellular pH has a strong influence on the microbial metabolism as well as product generation by microbes. Similarly, optimum temperature is also important as it affects the conversion efficiency of substrate into cell mass which affect the product formation, particularly when product is growth associated (Shah et al., 2010). Hence, in this study, pH and temperature were optimized to increase the L-asparaginase yield. The result suggests that as the pH increased, the enzyme production was also increased up to pH 8. Further increase in pH the enzyme production was declined (Fig. 1). The highest yield of enzyme was found to be at pH 8 (200.88µg/ml) that was considered as optimum pH. The temperature has significant effect on enzyme production as showed in Figure 2. The result suggests that as the temperature increased, the enzyme production was also increased up to 37^0C. Further increase in temperature the enzyme production was declined. The maximum production was achieved at 37^0C (188.88µg/ml). Our report of optimum temperature is in agreement with earlier report (Prasad Talluri et al., 2013).





The components of the fermentation medium should be supplied in an adequate quantity for growth, energy, building of cellular components and synthesis of fermented products where carbon and nitrogen sources play an important role (Shah et al., 2010). In present study we optimized glucose and yeast extract as source of carbon and nitrogen, respectively. The optimum glucose level was found to be 0.8% for maximum yield (3.83.88 µg/ml), while, below and above the 0.8%, the enzyme production was declined (Fig. 3). The optimum yeast extract level was found to be 1.5% for maximum yield (338.88µg/ml) while below and above the 1.5%, the enzyme production was declined (Fig. 4). These reports are correlated with previous study (Shah et al., 2010).





Conclusion:

In the present investigation, *Bacillus* sp. was isolated and optimized from agricultural soil. The result showed that optimum pH, temperature, glucose concentration as a carbon source and yeast extract as a source of nitrogen for L-asparaginase production by *Bacillus* sp. was 8, 37^0C, 0.8% and 1.5%, respectively. This clearly indicates that agriculture soil can provide a good source of L-asparaginase producing bacteria.

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