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Enhanced production of commercially important L-asparaginase by a newly isolated strain of *Bacillus* spp mlo5 from the gut of dragon fly

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ABSTRACT

Microbial secondary metabolites represent a large source of compounds endowed with indigenous structures and potent biological activities. An enteric bacteria of Dragon fly (*Libellula luctosa) Bacillus* spp ML05 showed significant L-Asparaginase activity. The maximum production of L-asparaginase was noticed at 37^{0}C, pH 6.5 and at mannitol (0.2 %), ammonium chloride (0.4 %) and dipotassium hydrogen phosphate (0.8 %) were ideal chemical sources for the enzyme. All selected factors showed impact on L-asparaginase enzyme production by this microbial strain. Significant improvement in enzyme production from 2.6 IU/ml to 4.2 IU/ml was noted under optimized environment.

KEYWORDS

Dragon fly;
Bacillus ML05;
L-asparaginase.

INTRODUCTION

Bacteria display a wide diversity of specialized interactions with eukaryotic hosts. Symbiotic associations with microorganisms are known for a broad range of animals, plants and other organisms[18,25] among which insects probably comprise the largest group in which symbiotic microorganisms are universally found[6,4]. Many insects harbour symbiotic microorganisms in their guts, body cavities or cells. Regardless of their obligate or facultative nature, these intracellular symbionts are generally potent producers of secondary metabolites. Insects are found to harbour many industrially important bacteria. Many of them are potent L-asparaginase producers which is an important chemotherapeutic agent. Tapping of such organisms for L-Asparaginase production in optimum medium will be worth attempting.

L-asparaginase received increased attention in recent years for its anticarcinogenic potential. Cancer cells differentiate themselves from normal cells in diminished expression of L-asparagine[27,17]. Hence, they are not capable of producing L-asparagine and mainly depend on the L-asparagine from circulating plasma pools[27]. Clinical trials indicate that this enzyme is also a promising agent in treating some forms of neoplastic cell disease in man[22]. The principal source of L-asparaginases for clinical trials is the bacterium *Escherichia coli*[2]. Although production and purification techniques have been developed, they generally provide a quantity of enzyme sufficient for only limited trials. Possibly, alternative sources of L-asparaginases could overcome the problem of antigenic reactions found in some patients[22].

L-asparaginase from two bacterial sources (*E.coli* and *Erwinia carotovora*) is currently in clinical use for the treatment of acute lymphoblastic leukemia[15]. It is also used for the treatment of pancreatic carcinoma[29]

and bovine lymphomosarcoma[21]. A partially purified L-asparaginase from *Aspergillus terreus* possesses the tumour property against Ehrlichs ascites in susceptible Swiss mice. From an observation of the toxicity and immunological responses, the enzyme may be non-toxic and have myelosuppressive/immunosuppressive[3].

Ever since *Escherichia coli* L- asparaginase antitumour activity was first demonstrated by Broome[5] and Mashburn and Wriston[19], its production using microbial systems has attracted considerable attention owing to their cost effective and eco friendly nature. A wide variety of microbial strains, such as *Erwinia aroideae*[16], *Serratia marcescens* (*Vibrio succino genes*)[23], *E.coli*[27,8], *Pseudomonas stutzeri*[17], *Pseudomonas aeruginosa*[1] and *Aspergillus tamari, Aspergillus terreus*[24] were screened for L-asparaginase producing potential[9,14]. In general, it is noticed that biochemical and enzyme kinetic properties like optimum pH, temperature, substrate specificity, inhibition pattern, etc., of microbial enzymes is well documented[28,10].

The aim of the present investigation is to search for new sources for L-asparaginase producing bacteria. Here the enteric cavity of Dragon fly (*Libellula luctosa*) was screened for L-asparaginase producing bacteria and find out the optimum nutritional and physiological requirements for maximum production of L-asparaginase.

MATERIALS AND METHODS

Isolation and screening of microorganism

The Dragon fly were collected from the garden of the Department of Environmental Sciences, Bharathiar University, Coimbatore and the enteric bacterial population were screened for the production of L-asparaginase.

Media composition

The growth medium used for L-asparaginase production composed of Peptone-0.5 %, Beef extract-0.5 %, Yeast extract-0.5% and L-asparagine-0.1%. The pH of the medium was adjusted to pH 6.5 and was autoclaved at 121^{0}C for 15 minutes.

L-asparaginase assay

L-asparaginase activity was determined by measuring the amount of ammonia formed by nesslerization[28]. A 0.5 ml sample of cell suspension, 1.0 ml of 0.1 M sodium borate buffer (pH 8.5) and 0.5 ml of 0.04 M L-asparagine solution were mixed and incubated for 10 min at 37^{0}C. The reaction was then stopped by the addition of 0.5 ml of 15% trichloroacetic acid. The precipitated protein was removed by centrifugation, and the liberated ammonia was determined by direct nesslerization. Suitable blanks of substrate and enzyme-containing samples were included in all assays. The yellow colour was read in a Beckman DB-G spectrophotometer at 500 nm. One international unit (IU) of L-asparaginase is that amount of enzyme which liberate 1µmole of ammonia in 1 min at 37^{0}C.

Optimization of enzyme production

Optimum conditions required for maximum production of L-asparaginase were ascertained by subjecting the strains to different level of pH, temperature, agitation, medium strength, carbon, nitrogen and phosphate sources. The effect of pH, temperature and agitation on bacterial cell growth and L-asparaginase production from *Bacillus* sp. ML05 were carried out at pH 4 to 8, temperature at 20^{0}C, 25^{0}C, 30^{0}C, 37^{0}C and at different agitation speed ranging from static, 100 rpm to 200 rpm. To investigate the effects of various carbon sources on L-asparaginase production, *Bacillus* sp. ML05 strain was grown in different media containing sucrose, maltose, fructose, lactose, glycerol, starch, galactose, xylose, arabinose, sorbitol and mannitol at 0.2, 0.4, 0.6 and 0.8 %. The effects of various nitrogen sources on L-asparaginase production in *Bacillus* sp. ML05 strain was investigated using different media containing gelatin, glycine, ammonium chloride, ammonium nitrate, ammonium sulphate, ammonium dihydrogen phosphate, sodium nitrate, monosodium glutamate, yeast extract and potassium nitrate at 0.2, 0.4, 0.6 and 0.8 %. The effects of various phosphate sources on L-asparaginase production was analyzed in *Bacillus* sp. ML05 strain using different media containing calcium phosphate, tricalcium phosphate, potassium phosphate, sodium phosphate and dipotassium hydrogen phosphate at 0.2, 0.4, 0.6 and 0.8 %. The L-asparaginase production was evaluated by diluting the media at different concentration of 25, 50, 75 and 100 percent in both conventional and modified medium.

RESULTS AND DISCUSSION

Out of the 15 isolates, *Bacillus* sp ML05 exhibited the maximum production of L-asparaginase. Therapeutic enzymes are gaining importance mainly because of their broad variety of specific uses such as oncolytics, thrombolytics or anticoagulants and as replacements for metabolic deficiencies. Biological production of any enzyme is a highly complex process, which involves several catalytic reactions and regulatory parameters at environmental and biochemical levels[11]. To maximize any enzyme production by isolated microbial organism, the basic need is to have preliminary information on growth conditions and its associated enzyme[12].

Effect of pH on L-asparaginase production

The optimum enzyme production and cell density was maximum at pH 6.5 and production rate declined with increasing and decreasing pH (Figure 1). Enzyme production was observed till pH 8 but comparatively low with the optimal pH values. The culture pH strongly affects many enzymatic process and transportation of various components across the cell membrane[20].

Effect of temperature and agitation on L-asparaginase production

The temperature and aeration are the main factors that govern the enzyme production. Ray et al.[24] had shown that temperature can regulate the synthesis and secretion of enzymes by microorganisms. The growth of microorganisms and enzyme production is significantly affected by aeration and agitation[13]. In the present study, enzyme production was found to enhance considerably under static condition as it greatly influenced the availability of nutrients to the organism at 37^{0}C (Figures 2 and 3).

Effect of carbon, nitrogen and phosphate source on L-asparaginase production

Cell growth and L-asparaginase production are usually stimulated by supplying carbon, nitrogen and phosphate sources. The optimization of carbon, nitrogen and phosphate sources showed that the maximum enzyme activity at mannitol, ammonium chloride and dipotassium hydrogen phosphate repressed enzyme production with 0.2 %, 0.4 % and 0.8% concentrations (Figures 4-6). There are several reports showing that different carbon, nitrogen and phosphate sources have different influences on enzyme production by different strains[7].

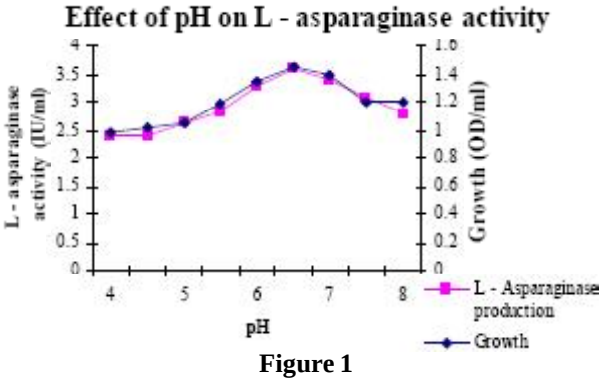
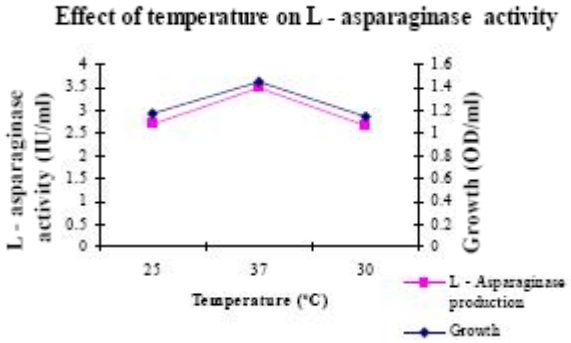


Figure 1



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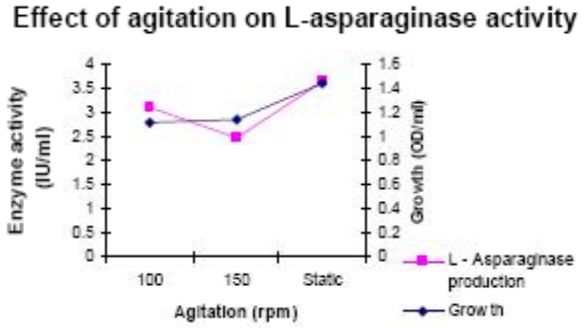

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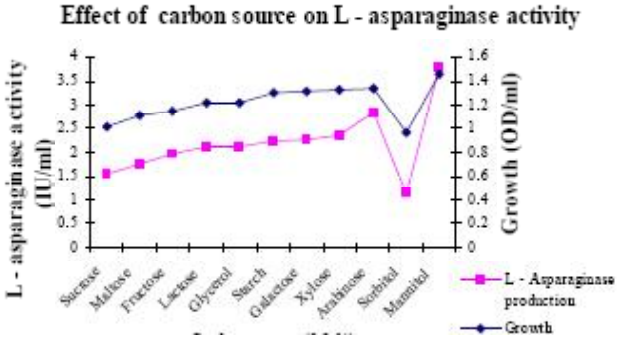

Figure 4

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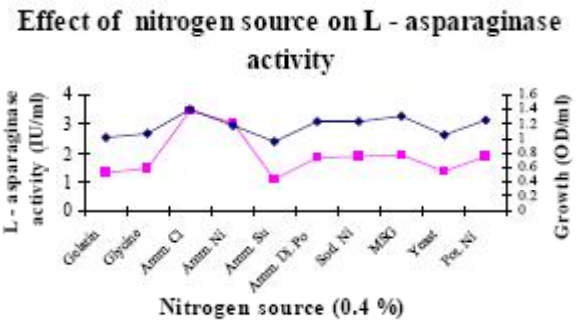

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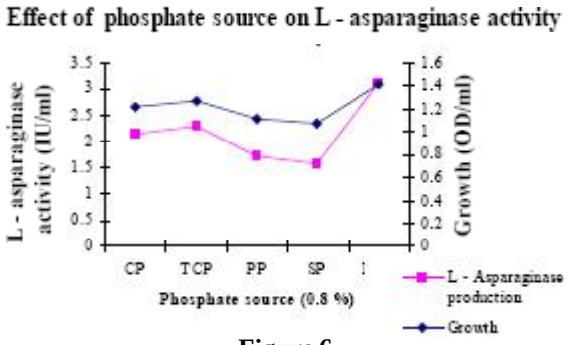
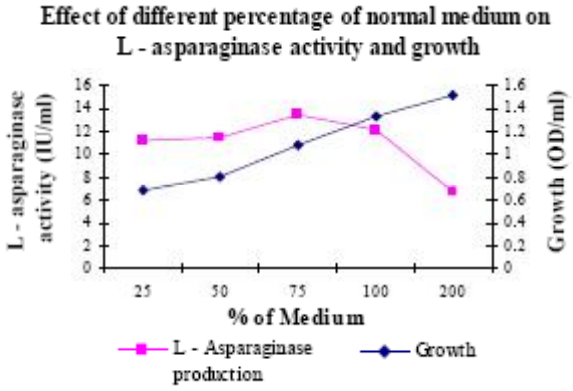


Figure 6



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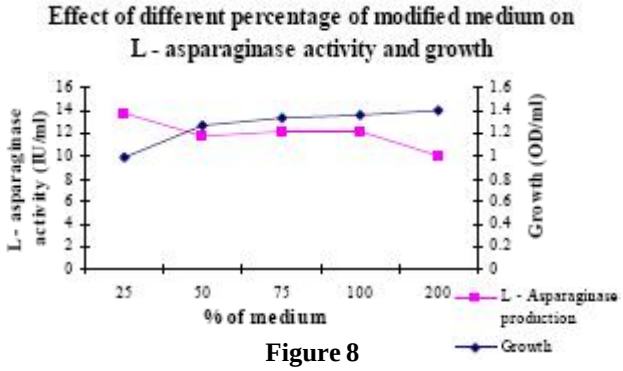

Figure 8

Effect of different medium strength on L-asparaginase production

The conventional medium at 75 % exhibited maximum L-asparaginase production (2.6 IU/ml) with minimum cell density whereas the L-asparaginase production was high (4.2 IU/ml) at 25 % in modified medium with minimum cell growth. (Figures 7 and 8)

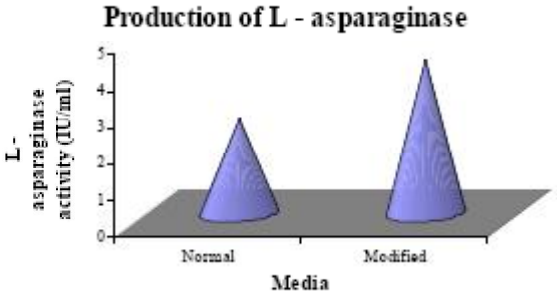

Figure 9

The production of 4.2 IU/ml L-asparaginase was achieved when *Bacillus* sp. ML05 was grown in pH 6.5 at 37^{0}C under static conditions with mannitol (0.2%), ammonium chloride (0.4%) and dipotassium hydrogen phosphate (0.8%) at 25 % medium strength. (Figure 9)

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