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Microbial fermentation biotechnology of cooked chicken bone novel substrate for L-asparaginase production

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Abstract

L-asparaginase is an efficient anti-cancer enzyme due to its remarkable property in hydrolyzing essential amino acid of acute lymphoblastic leukemia cancer (L-asparagine) into aspartic acid and ammonia. Various sources of L-asparaginase had been identified including extraction from animal or plant as well as through microbial fermentation. Generally, researchers preferred to generate L-asparaginase by engaging microbe as the L-asparaginase producer because the abundant amount of L-asparaginase can be harvested in an affordable manner. The present study aimed at screening, optimization, and purification of microbial production L-asparaginase in presence of cooked chicken bone wastes as substrate. Different controlling parameters were studied including physiological (incubation period and temperature, initial pH-value, and substrate concentration), nutritional (carbon and nitrogen sources) and microbial parameters (inoculum sizes). As a result, the highest amount of L-asparaginase was harvested when the fermentation was incubated at 40 °C for 2 days at pH 9 in presence of 1% w/v of cooked chicken bone waste as a sole substrate. Besides that, starch and ammonium chloride were discovered as the best-supplemented carbon and nitrogen sources respectively when 12% v/v of *Escherichia coli* ATCC 10536 suspension was inoculated. The harvested L-asparaginase has proceeded with a series of purification and the specific activity achieved after partial purification was 0.549 IU/mg. In conclusion, optimization of controlling parameters as well as supplementation of cooked chicken bone as substrate capable to further enhance the production of L-asparaginase.

Keywords: L-asparaginase; Cooked chicken bone; *E. coli* ATCC 10536; Optimization; Characterization

1. Introduction

L-asparaginase is scientifically known as L-asparagine amidohydrolase. It is known as a great anti-leukemia enzyme due to its remarkable property in hydrolyzing L-asparagine into aspartic acid and ammonia [1] where L-asparagine is an essential amino acid in the proliferation of leukemic cell as explained

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[2]. Besides that, L-asparaginase enzyme is also utilized as pre-treatment aid in the food industry. Food with the high content of starch such as bread and potato will undergo Maillard reaction when they are exposed to the high temperature such as baking and frying. Although Maillard reaction is responsible for smell, color, and taste of the food it will produce acrylamide compound at the end of the reaction and this compound is known to be carcinogenic in long term consumption. The formation of acrylamide is solely caused by the reaction between reducing sugars and asparagine at high temperature [3]. As a result, it is necessary to pre-treat the starchy food ahead in order to prevent the formation of acrylamide. In addition, L-asparaginase enzyme is also immobilized on various carriers in order to fabricate L-asparaginase biosensor. As a result, L-asparaginase is highly demanded by various industries due to its significant property [4]. Generally, researchers preferred microbial fermentation as the source of L-asparaginase compared to extraction from animal and plant because microbial fermentation not only offers a great alternative to harvest a vast amount of L-asparaginase in the most economical way [5] but the outcome yield can be further improved by optimizing its controlling parameters. In addition, a wide diversity of microbe such as bacteria, fungus, actinomycetes as well as algae can be engaged as L-asparaginase producer and among all, bacteria are widely studied by researchers because it is highly stable isolate as claimed [6]. Besides that, El-Bessoumy [7] clarified Gram negative bacteria tend to perform better in the production of L-asparaginase as he discovered *Pseudomonas aeruginosa* 50071 is an excellent L-asparaginase producer. On the other hand, excessive extraction of L-asparaginase from animal and plant in fulfilling the enzyme demand will interrupt the balance of the food chain cycle.

Significantly, microbial fermentation can be further enhanced by the addition of substrate either with natural or waste forms. Generally, the researcher's favored waste as a substrate because it is highly available and cheaper compared to natural products as well as reuse and reduces the environmental waste. Aside from that, utilization of waste also can cut the risk of contamination. Many substrates were supplemented as corn cob, squid pen, sugar cane industry effluent and cotton seed waste as a substrate, respectively in enhancing the microbial production of L-asparaginase [8–11].

In chicken, the content of bone ash in dry matter is 47.7%–70.3% [12–16]. The bone ash in turkeys [17] and geese [16] is about 70%. In poultry, the content of crude fat is 26–31% of the dry matter, in a buzzard 16.7% and in a falcon 17.7%. Contrary to mammals the content of crude fat remains more or less constant during growth in chicken [18]. The skeleton contains over 99% of the calcium and about 80% of the phosphorus in the body in a ratio of 2:1 [16]. A calcium content of 200 g/kilo to 212 g/kg fat-free dry matter and a phosphorus content of 102 g/kilo to 108 g/kg fat-free dry matter is found in the bones of different pet bird species. Therefore, the aim of the study is to identify cooked chicken bone wastes as a new substrate (1st report on this study) which can be easily fermented by microbial isolates in generating L-asparaginase. And so, microbial screening, optimization, partial purification and characterization of L-asparaginase were studied in the present paper.

2. Materials and methods

2.1. Materials

M9 minimal media purchased from Sigma–Aldrich Chemical Co, Czapek Dox's and nutrient media (Oxoid). All buffers and other reagents were of analytical grade.

2.2. Substrate preparation

Cooked chicken bones (CCB) waste was collected from the cafeteria at Universiti Malaysia Pahang (UMP), Gambang, Kuantan area, Pahang, Malaysia. The sample was thoroughly cleaned under running water and dried at 45 °C overnight. Then, the sample was grounded into powder form using grinding machine (Retsch). The substrate was exposed to ultraviolet ray for 10 min before being supplemented as microbial substrate.

2.3. Inoculum preparation

Approximately thirty-five (35) microbial isolates were used in this survey (31 bacterial and 4 fungal isolates) were collected from Faculty of Industrial Sciences & Technology (FIST) lab, UMP (previously isolated from different sources and purified). One loopful of bacteria was aseptically inoculated into 50 ml of nutrient broth media (LAB M, United Kingdom) whereas one loopful of fungal isolates was aseptically spread on potato dextrose agar (PDA). Both bacterial and fungal isolates were incubated at 37 °C for 48 h and 1 week respectively.

2.4. L-asparaginase production media preparation

M9 minimal medium (standard) was employed by bacterial isolates whereas fungal isolates utilized Dox's

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