

Full Length Research Paper

Production and optimization of L-asparaginase by *Bacillus* sp. KK2S4 from corn cob

Makky E. A.*, Jee Jian Ong, Md. Rezaul Karim and Lee C. M.

Faculty of Industrial Sciences and Technology, University Malaysia Pahang, Tun Razak Highway, 26300 Kuantan, Pahang, Malaysia.

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L-Asparaginase (L-asparagine amido hydrolase, E.C.3.5.1.1) is an important enzyme which has anti-tumor properties. This paper describes the production and optimization of L-asparaginase by *Bacillus* sp. KK2S4 utilization of ground corn cob waste as substrate to reduce and manage the waste in our environment. Thirty-nine (39) bacterial isolates were screened for their L-asparaginase productivity and protein content. Only *Bacillus* sp. KK2S4 was identified before and selected as the most potent isolate. Modified M9 (MM9) minimal complete medium (glucose free) was used as production media for enzyme production. The highest enzyme production was detected at pH 5.0 in presence of lactose and NaNO₃ as the best carbon and nitrogen sources with high activity increasing percentage of 1058.9, 377.3 and 566.7%, respectively.

Key words: L-Asparaginase production, corn cob waste, enzyme optimization, anti-tumor agent.

INTRODUCTION

Many enzymes have been used as drugs, like wise L-asparaginase attracted much attention because of its use as effective therapeutic agent against lymphocytic leukemia and other kinds of cancer in man (Krasotkina et al., 2004). This enzyme is widely distributed, being found in animal, microbial and plant sources as well as large number of microorganisms that include *Erwinia carotovora*, *Pseudomonas stutzeri* (Mannan et al., 1995), *Pseudomonas aeruginosa* (Abdel-Fatteh and Olama, 2002) and *Escherichia coli* (Qin and Zhao, 2003; Jain et al., 2012). It has been observed that eukaryotic microorganisms like yeast and fungi have a potential for asparaginase production (Pinheiro et al., 2001). L-Asparaginases (EC 3.5.1.1) hydrolyze L-asparagine to L-aspartate (also known as L-aspartic acid) and ammonia as shown in Figure 1 (Rani et al., 2012; Verma et al. 2007; Savitri and Azmi, 2003; Borek and Jaskólski, 2001). The effects of municipal solid waste compost and decomposed cow manure on L-asparaginase activities in sub-

merged rice soil have been studied. L-Asparaginase and L-glutaminase activities were reported higher in decomposed cow manure, as compared to municipal solid waste compost treated soils due to higher amount of organic materials like water soluble organic carbon, carbohydrate and mineralized nitrogen in decomposed cow manure (Bhattacharya et al., 2007). This paper deals with the production and optimization of L-asparaginase from *Bacillus* sp. using corn cob waste as a substrate. There were no reports on production and optimization of L-asparaginase by using corn cob substrate.

MATERIALS AND METHODS

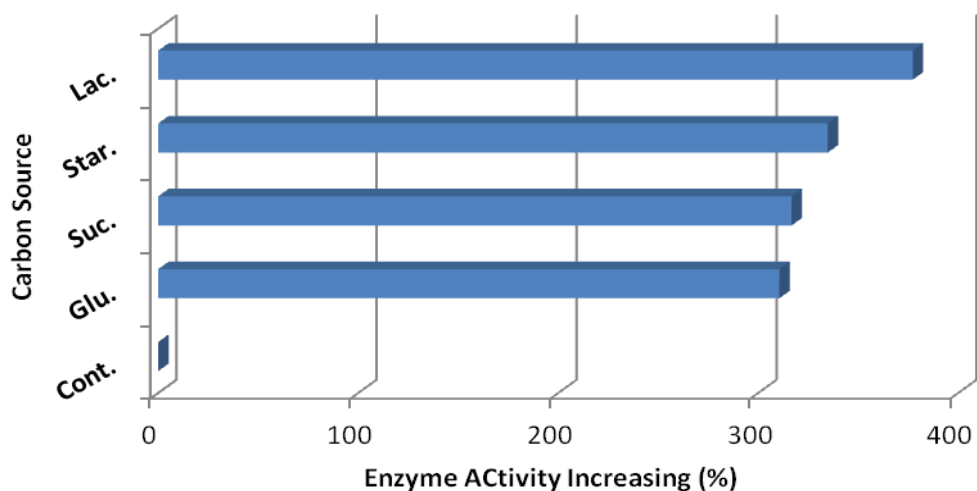
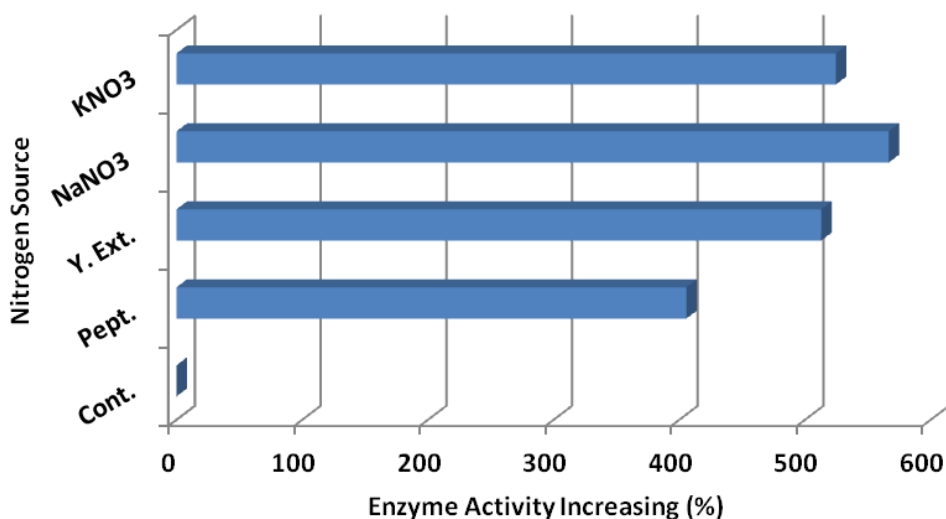
Isolation of bacterial isolates

Nutrient agar (NA) was prepared for bacterial isolation using serial dilution method from different sources (soil, water and food samples) collected from different areas of Kuantan, Pahang, Malaysia.

*Corresponding author. E-mail: essam22001@gmail.com. Tel.: (+6)09-5492454. Fax: (+6)09-5492766.

Table 1. Selection of most potent isolate using treated corn cob waste at 37°C.

Isolate code	Asparaginase activity (450 nm)	Protein content (mg/ml)
	Mean \pm SD	Mean \pm SD
Control	0.098 \pm 0.010	0.290 \pm 0.007
KK2S4	0.261 \pm 0.014	0.569 \pm 0.000
KK2S5	0.239 \pm 0.005	0.590 \pm 0.004
PS1	0.233 \pm 0.005	0.494 \pm 0.008
CL14	0.185 \pm 0.005	0.533 \pm 0.003

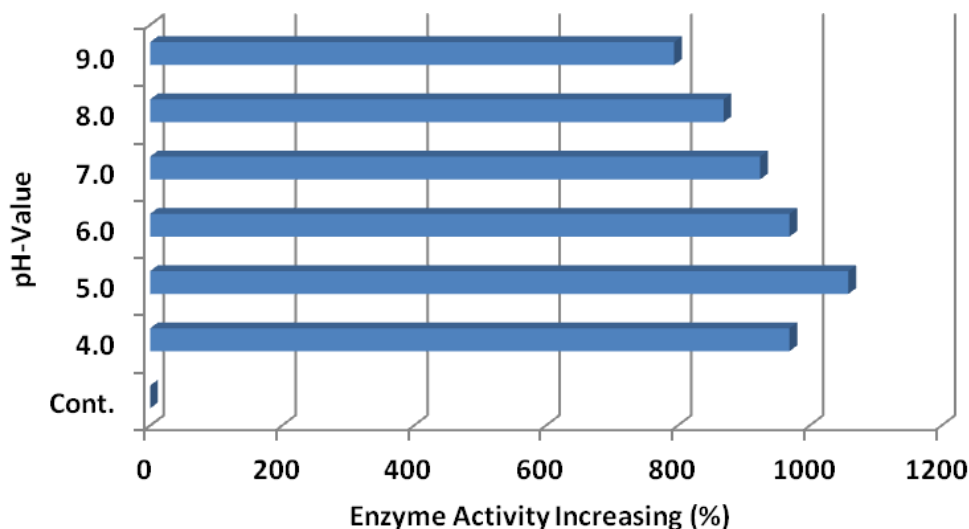
**Figure 2.** Graph of different carbon sources used showing activity increasing percentage.**Figure 3.** Graph of different nitrogen sources used showing activity increasing percentage.

that glucose and sucrose can inhibit the enzyme production; especially, glucose considered as a repressor for L-asparaginase production in bacteria may be due to

some catabolites repression and inhibition of the components involved in lactate transport and lactate stimulated L-asparaginase synthesis (Kenari et al., 2011).

Table 2. Optimization of anti-cancer productivity from corn cob waste by KK2S4 at 37°C.

Parameter	Asparaginase activity (U/ml)	Protein content (mg/ml)	Specific activity (U/mg)	Activity increasing (%)
	Mean \pm SD	Mean \pm SD		
Different carbon source				
Control	0.278 \pm 0.009	1.052 \pm 0.007	0.084	0.0
Glucose	0.271 \pm 0.006	1.782 \pm 0.043	0.152	310.6
Sucrose	0.275 \pm 0.009	1.777 \pm 0.028	0.155	316.7
Starch	0.287 \pm 0.002	1.812 \pm 0.004	0.158	334.8
Lactose	0.315 \pm 0.014	2.012 \pm 0.010	0.157	377.3
Different nitrogen sources				
Control	0.278 \pm 0.009	1.052 \pm 0.007	0.065	0.0
Peptone	0.349 \pm 0.009	3.190 \pm 0.019	0.109	405.8
Yeast extract	0.423 \pm 0.027	3.200 \pm 0.023	0.132	513.0
Sodium nitrate	0.460 \pm 0.008	2.132 \pm 0.052	0.216	566.7
Potassium nitrate	0.431 \pm 0.003	2.183 \pm 0.004	0.197	524.6
Different pH-values				
Control	0.452 \pm 0.006	0.955 \pm 0.002	0.059	0.0
4.0	0.599 \pm 0.015	1.001 \pm 0.027	0.598	969.6
5.0	0.649 \pm 0.001	1.288 \pm 0.018	0.504	1058.9
6.0	0.599 \pm 0.012	1.272 \pm 0.019	0.471	969.6
7.0	0.574 \pm 0.009	1.804 \pm 0.008	0.318	925.0
8.0	0.543 \pm 0.009	2.094 \pm 0.023	0.259	869.6
9.0	0.501 \pm 0.018	2.240 \pm 0.024	0.224	794.6

**Figure 4.** Graph of different pH-values showing activity increasing percentage.

The acidic nature of fermentation medium could inhibit L-asparaginase biosynthesis (Narayana et al., 2008). In a study using *Bacillus* strain (DKMBT10), the purified L-asparaginase has greater specific activity with glucose as

carbon source (1.12 U/mg) when compared with maltose (1.05 U/mg) (Moorthy et al., 2010). In other reports that used *Bacillus cereus* MNTG-7, tapioca starch was found to be the best carbon source with positive coefficient

(6.390) (Sunitha et al., 2010). This could be due to the different strain of *Bacillus* sp. In the present study, nitrogen catabolite repression on enzyme formation was absent in *Bacillus* sp. With isolate, it was shown that all nitrogen sources gave better results than control. In contrast, most studies indicate that yeast extract is the best nitrogen source. According to Kenari et al. (2011), yeast extract showed the best nitrogen source for *E. coli* ATCC (11303) with 0.15 U/ml. Also found to be the most significant variable for *E. carotovora* MTCC 1428 (Deokar et al., 2010). Yeast extract is the best organic nitrogen source for *Fusarium equiseti* during L-asparaginase production under solid state fermentation (Hosamani and Kaliwal, 2011). *Streptomyces albidoflavus* also required yeast extract as nitrogen source for maximum L-asparaginase production (Narayana et al., 2008). In the present study, the result has proven that *Bacillus* sp. can produce maximum L-asparaginase enzyme at acidic environment. As the pH increases beyond pH 5.0, the L-asparaginase productivity also decreases consistently. This shows that alkaline condition is not suitable for *Bacillus* sp. When grown on corn cob as a substrate to yield optimal L-asparaginase enzyme. A study which has almost similar result was carried out using deseeded carob pod as substrate. L-Asparaginase produced by *Aspergillus terreus* KLS2 was found to be highest at pH 4.5 with L-asparaginase activity of 5.210 IU after 72 h of submerged fermentation process (Siddalingeshwara and Lingappa, 2010). For *S. albidoflavus*, as pH increases from pH 5.0, the L-asparaginase activity also increases. After pH 7.5, the L-asparaginase activity started to decrease (Narayana et al., 2008).

In conclusion, this is the first report of study that used *Bacillus* sp. and was selected to be the most potent microbial isolate for L-asparaginase (anti-cancer agent) production using corn cob as the sole carbon source at 37°C for 48 h incubation period. The enzyme was successfully enhanced and exhibited higher productivity at pH 5.0 in the presence of 20% lactose, sodium nitrate, 200 ppm asparagine and corn cob in the MM9 minimal complete medium as production media.

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