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Efficient production of L-asparaginase from *Bacillus licheniformis* with low-glutaminase activity: Optimization, scale up and acrylamide degradation studies

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HIGHLIGHTS

- Production of L-asparaginase from *Bacillus licheniformis* and its statistical optimization.
- High yields of enzyme obtained i.e. 32 IU/ml in 18 h after statistical optimization.
- Optimized process yielded 30 IU/ml of enzyme in 15 h is obtained in 30 L fermenter.
- L-Asparaginse produced by *B. licheniformis* is free of glutaminase activity.
- L-Asparaginase produced was efficiently able to degrade polyacrylamide.

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ABSTRACT

L-Asparaginase has potential as an anti-cancer drug and for prevention of acrylamide formation in fried and baked foods. Production of the enzyme by *Bacillus licheniformis* (*RAM-8*) was optimized by process engineering using a statistical modeling approach and a maximum yield of 32.26 IU/ml was achieved. The L-asparaginase exhibited glutaminase activity of only 0.8 IU/ml and would therefore be less prone to cause the side effects associated with asparaginase therapy compared to enzyme preparations with higher glutaminase activities. When production was carried out in a 30-L bioreactor, enzyme production reached 29.94 IU/ml in 15 h. The enzyme inhibited poly-acrylamide formation in 10% acrylamide solution and reduced acrylamide formation in fried potatoes by 80%.

1. Introduction

L-Asparaginase (L-asparagine amidohydrolase E.C.3.5.1.1) can be used for the treatment of acute lymphoblastic leukemia (ALL) (Warangkar and Andkhobragade, 2008; Siddalingeshwara and Kattimani, 2010) since it catalyzes the irreversible conversion of L-asparagine to L-aspartate and ammonia under physiological conditions in the blood stream (Lubkowski et al., 1996), thereby depriving malignant lymphoblastic cells of an essential nutrient (Ohnuma et al., 1970). The enzyme can also be used to reduce the formation of acrylamide in fried and oven-cooked foods especially in potato chips (Rosen and Hellenäs, 2002; Tareke et al., 2002). Since, acrylamide formation in heated foods is mainly due to the reaction of free asparagine and reducing sugars (Ciesarova et al., 2006), deamination of asparagine prevents acrylamide formation (Ciesarova et al., 2006).

L-Asparaginase is widely distributed among plants, animals and microorganisms (Wriston and Yellin, 1973). Microorganisms known to produce this enzyme include *Escherichia coli*, *Erwinia carotovora* (Warangkar and Andkhobragade, 2008), *Enterobacter aerogenes* (Mukherjee et al., 2000), *Corynebacterium glutamicum* (Mesas et al., 1990), *Pseudomonas aeruginosa* (El-Bessoumy et al., 2004), *Candida utilis* (Kil et al., 1995), *Aspergillus tamari* (Sarquis et al., 2004), *Aspergillus terreus* (Sarquis et al., 2004), *Staphylococcus aureus* (Prakasham et al., 2007) and *Thermus thermophilus* (Prista and Kyridio, 2001). The principle sources of L-asparaginase for therapeutic uses have been *E. carotovora* and *E. coli* (Duval et al., 2002; Warangkar and Andkhobragade, 2009). Although production and purification techniques have been developed for L-asparaginase recovery from the bacteria, enzyme yields have been low (Kenari et al., 2011). Also, cancer treatment with asparaginases

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