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Review

Optimization and purification of L-asparaginase from fungi: A systematic review

Paula Monteiro Souza[a,*], Marcela Medeiros de Freitas[a], Samuel Leite Cardoso[a], Adalberto Pessoa[b], Eliete Neves Silva Guerra[c], Pérola Oliveira Magalhães[a,*]

[a] Department of Pharmacy, Health Sciences School, University of Brasilia, Brazil
[b] Department of Biochemical and Pharmaceutical Technology, University of São Paulo, Brazil
[c] Laboratory of Oral Histopathology, Faculty of Health Sciences, University of Brasilia, Brasília, Brazil

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ABSTRACT

The purpose of this systematic review was to identify the available literature of the L-asparaginase producing fungi. This study followed the Preferred Reporting Items for Systematic Reviews. The search was conducted on five databases: LILACS, PubMed, Science Direct, Scopus and Web of Science up until July 20th, 2016, with no time or language restrictions. The reference list of the included studies was crosschecked and a partial gray literature search was undertaken. The methodology of the selected studies was evaluated using GRADE. Asparaginase production, optimization using statistical design, purification and characterization were the main evaluated outcomes. Of the 1686 initially gathered studies, 19 met the inclusion criteria after a two-step selection process. Nine species of fungi were reported in the selected studies, out of which 13 studies optimized the medium composition using statistical design for enhanced asparaginase production and six reported purification and characterization of the enzyme. The genera *Aspergillus* were identified as producers of asparaginase in both solid and submerged fermentation and L-asparagine was the amino acid most used as nitrogen source. This systematic review demonstrated that different fungi produce L-asparaginase, which possesses a potential in leukemia treatment. However, further investigations are required to confirm the promising effect of these fungal enzymes.

1. Introduction

The major types of cancers in children ages 0–14 years are acute lymphocytic leukemia (ALL), brain and other central nervous system tumors, and neuroblastoma, which are expected to account for more than half of new cases in 2016 (NCI, 2016). The inhibitory action of guinea pig serum on the cells of three transplantable mouse and rat lymphomas *in vivo* was described years ago, which was later discovered that the L-asparaginase activity of guinea pig serum is responsible for the anti-lymphoma effect (Broome, 1961). Asparaginase is listed in the 19th WHO List of Essential Medicines and WHO Model List of Essential Medicines for Children as a cytotoxic and adjuvant medicine for acute lymphoblastic leukemia (WHO, 2015). Among other drugs such as vincristine and corticosteroid, L-asparaginase is used as a remission induction chemotherapy standard treatment option for newly diagnosed ALL. It is also used among dexamethasone and methotrexate with leucovorin rescue as a central nervous system-directed systemic chemotherapy prophylaxis for standard-risk and high-risk ALL although it does not penetrate into cerebrospinal fluid (CSF) itself, but leads to CSF asparagine depletion (NCI, 2016).

L-asparaginase (EC.3.5.1.1; L-asparagine aminohydrolase) catalyzes the deamination of L-asparagine to L-aspartate and ammonia. Neoplasic cells cannot synthesize L-asparagine unlike normal cells due to the low expression or absence of the L-asparagine synthetase gene, therefore they obtain the required asparagine from circulating pools. This enzyme is widely distributed in nature, being found not only in microorganisms, but also in plants and tissues (liver, pancreas, brain, ovary or testes, kidneys, spleen and lungs) of several animals like fishes, mammals and birds. However, microbes are a better source than animals or plants, considering their ability to grow easily on rather simple and inexpensive substrates (Lopes et al., 2015). There are two different types of bacterial asparaginases, which differ significantly in their affinities for L-asparagine. Type I L-asparaginases are cytoplasmatic enzymes that show low affinity to asparagine, while type II L-asparaginases are located in the periplasmic space with high affinity to substrate. Only type II asparaginases have been used as therapeutic agent, because the

* Corresponding authors.
E-mail addresses: paulasouza@unb.br (P.M. Souza), perolamagalhaes@unb.br (P.O. Magalhães).

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enzyme's antitumor activity is related to their affinity for L-aspargine. The L-asparaginases from *Escherichia coli* and *Erwinia*, which have high L-asparagine specificity, are the only sources available for clinical applications against ALL (Yun et al., 2007). Industrialized enzyme preparations include *E. coli* derived asparaginase Crastinin®, Elspar®, Kidrolase®, Leunase®, Asparaginase medac™; *Erwinia* derived asparaginase Erwinase®; PEGylated *E. coli* asparaginase Oncaspar® (Pieters et al., 2011) and recombinant *E. coli* asparaginase, Spectrila®. However, adverse effects such as anaphylactoid reactions have been reported in children with leukemia and lymphoma when asparaginase from *E. coli* and *Erwinia* was administered (Evans et al., 1982). The hypersensitivity reactions occurs in approximately 60% of patients during therapy of L-asparaginase from *E. coli*, even with the PEG asparaginase, and it ranges from allergic reactions, anaphylactic shock, coagulation disorder, edema, rash, broncospasm and can also lead hepatotoxicity, pancreatitis, hyperglycaemia and their related reactions. These side effects of L-asparaginase could be due to its L-glutaminase activity. This activity results in some reduction of plasma L-glutamine level, which is an amino group donor for the enzyme L-asparagine synthetase for *de novo* biosynthesis of L-asparagine. Therefore, the decreased glutamine level and the asparagine level reduction contribute to the therapeutic effect of L-asparaginase. Moreover, the allergic reactions are due the production of anti-asparaginase antibody and are responsible for resistance in asparaginase therapy, besides increasing the asparagine levels in blood (Keating et al., 1993; Shrivastava et al., 2015). Furthermore, some industrialized asparaginases have been discontinued while others are not available in all countries, making the production of this enzyme important to meet the global demand for medicines used as treatment for ALL.

In this scenario, it is important to find new sources of L-asparaginase producing microorganisms that can avoid undesired side effects obtained from bacterial L-asparaginase. These findings should guide researchers towards a direct approach to produce and purify high yields of the enzyme from eukaryotic microorganisms such as fungi in an attempt to reduce such side effects and supply the global market with ALL treatment. Therefore, the purpose of this systematic review was to identify the available literature of the L-asparaginase producing fungi.

2. Methods

This systematic review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) Checklist (Moher et al., 2009). The protocol was not registered because it is a systematic review of *in vitro* studies. This type of systematic review is not eligible for inclusion in the International Prospective Register of Systematic Reviews (PROSPERO).

2.1. Eligibility criteria

2.1.1. Inclusion criteria

Articles that focused on optimization for asparaginase production, purification and characterization were eligible for inclusion. Studies that evaluated optimization of enzyme production using statistical experimental designs and complete purification with all characterization data (temperature, pH, kinetic and stability) were considered.

2.1.2. Exclusion criteria

The following studies were excluded: (1) Papers with molecular studies of L- asparaginase gene without enzyme production data; (2) Studies that did not use statistical design to optimize L-asparaginase production; (3) Studies that only reported L-glutaminase activity; (4) Papers that only conducted screening studies or did not measure L-asparaginase activity; (5) Papers with *in vivo* studies; (6) Studies that did not purify the enzyme completely or did not characterize the purified enzyme; (7) Reviews, letters, personal opinions, book chapters and conference abstracts.

2.2. Information sources and search strategy

Detailed individual search strategies for each of the following bibliographic databases were developed: LILACS, PubMed, Science Direct, Scopus, and Web of Science (Appendix A). A partial gray literature search was performed using Google Scholar, OpenGrey and ProQuest Dissertations & Theses Global. The search included all articles published up until July 20, 2016, across all databases with no time restrictions. In addition, the reference lists of selected articles were hand screened for potentially relevant studies that could have been missed during the electronic database search, and experts in the field were consulted. Duplicated references were removed using reference manager software (EndNote, Thomson Reuters).

2.3. Study selection

The study selection was completed in two phases. In phase one, two authors (P.M.S. and S.L.C.) independently reviewed the titles and abstracts of all the references. These authors selected studies that appeared to meet the inclusion criteria based on their titles and abstracts. A third author (M.M.F.) was consulted when disagreements emerged between the two initial evaluators. Any studies that did not fulfill the inclusion criteria were discarded. In phase two, two authors (P.M.S. and S.L.C.) read all the full-text articles and excluded those which were not in agreement with the inclusion criteria. The three authors (P.M.S., S.L.C. and M.M.F.) independently reviewed all full-text articles. Any disagreement in either phase was resolved by discussion and mutual agreement among the three reviewers.

2.4. Data collection process and data items

Two authors (P.M.S. and S.L.C.) collected the required information from the selected articles. A third author (M.M.F.) independently checked the data extraction tables for accuracy and detail. Again, any disagreement in either phase was resolved by discussion and mutual agreement among the three authors. For all of the included studies, the following information was recorded: year of publication, author(s), country and site, fungus species, growth conditions, asparaginase activity, purification steps, enzymatic characterization data and main conclusions.

2.5. Risk of bias in individual studies

The methodology of selected studies was evaluated using GRADE, a tool for the quality assessment of the evidence of studies (Guyatt et al., 2011). The GRADE tool was adapted to *in vitro* studies, according to Borges et al. (2017), given that no specific quality assessment method was developed for this type of study. P.M.S. and S.L.C. scored each item as 'high', 'moderate', 'low', or 'very low' quality and independently assessed the quality of each included study. Disagreements were resolved by a third reviewer (M.M.F.).

2.6. Summary measures

Asparaginase production, optimization using statistical design, purification and characterization were the main evaluated outcomes.

3. Results

3.1. Study selection

In phase 1 of study selection, 1686 citations were identified across the five electronic databases. After the duplicate articles were removed, remained 1450 citations. Comprehensive evaluation of the abstracts was completed and 1370 articles were excluded, so 80 articles remained after phase 1. Twenty-three articles were identified using