International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 2, 2016

Original Article

PRODUCTION AND PURIFICATION OF ANGIOTENSIN-CONVERTING ENZYME INHIBITOR BY SELECTED BACTERIAL STRAIN FOR CANCER THERAPY

LIDA EBRAHIMI[1], K. A. AYUB NAWAZ[1], K. G. KIRAN[1], S. DURAIRAJU NISSHANTHINI[1], MUTHUSAMY PALANISWAMY[2], JAYARAMAN ANGAYARKANNI*[1]

[1]Department of Microbial Biotechnology, Bharathiar University, Coimbatore (T. N) 641046, India, [2]Department of Microbiology, Karpagam University, Coimbatore (T. N) 641021, India
Email: angaibiotech@buc.edu.in

Received: 09 Aug 2015 Revised and Accepted: 19 Dec 2015

ABSTRACT

Objective: The present study was planned to explore safer, innovative and economic Angiotensin-converting enzyme inhibitors (ACEi) from beef extract by the action of a proteolytic *Micrococcus luteus*. Cytotoxicity of the stable peptide was predicted using MCF-7 cell line *in vitro.*

Methods: ACEi was purified by sequential steps of ethanol precipitation, ion exchange column chromatography (MonoQ) and gel filtration column chromatography (Sephadex G25). The apparent molecular mass was determined by SDS-PAGE. The anticancer property was analyzed by studying the cytotoxicity effects of angiotensin converting enzyme inhibitor using Breast cancer MCF-7 cell lines

Results: The peptide was purified and molecular mass was determined as 4.5 kDa. The IC_{50} value of peptide was found to be 59.5 µg/ml. The DNA fragmentation was not observed in the treated cells. The purified peptide has demonstrated to induce apoptosis of cancer cell. The results proved that the peptide has the ability to be used for cancer therapy.

Conclusion: The presence of ACE inhibition activities in the fermentation of beef extract using *Micrococcus luteus* has been investigated. The Peptide has been determined as an active compound that inhibited the activity of ACE. These properties indicate the possibilities of the use of purified protein as a potent anticancer agent.

Keywords: Angiotensin-converting enzyme inhibitors, *Micrococcus luteus*, Anti-proliferative, Anti-metastatic, MCF-7 cell line, Anticancer activity.

INTRODUCTION

Angiotensin-converting enzyme (ACE) is a dipeptide hydrolase that catalyses both the formation of the potent vasoconstrictor, angiotensin-II (Ang II), and the deactivation of bradykinin, a vasodilator peptide. Given the potential of Angiotensin II in containing the proliferation of the tumour, production of these inhibitors by bacteria could open new doors for anticancer therapy. The protective effect of ACEi cannot be attributed solely to the inhibition of AngII production. Rather, multiple mechanisms that are not yet fully understood could be responsible for the same. ACEi has also been reported to suppress vascular endothelial growth factor (VEGF), which is believed to play a major role in stimulating angiogenesis in human growth [1]. Although genotypic studies on ACE and the risk of cancer in humans have yielded contradictory results, the use of ACE inhibitors in experimental animal models has consistently indicated a protective effect of these drugs against tumor development. Perindopril, a well known ACEi used either alone or in combination with β interferon, was found to inhibit VEGF expression, endothelial cell migration and tubular formation in matrigel, thus proving its role in protection against tumor angiogenesis [2, 3]. Captopril, yet another ACEi, was also observed to attenuate tumor growth and angiogenesis of syngeneic fibrosarcoma when injected in rats [4].

In the recent years, several studies have identified peptides to act as inhibitors against ACE activity. ACE-inhibitory peptides have been discovered, isolated and purified from enzymatic hydrolyzates of different food proteins. Physiological and pharmacological effects of ACE inhibitory peptides derived from food proteins and their prospective applications in preventing hypertension, cancer and for therapeutic purposes have also been reported [5]. Various bioactive peptides including ACE-inhibitory or antihypertensive peptides, immunomodulatory, antioxidative, antimutagenic, anticancer peptides have been released from milk proteins, eggs, meat and fish as well as in different plant protein sources such as soy and wheat through microbial proteolysis [6-8]. The choice of the strain, which influences the release of effective bioactive peptides, is one of the most effective ways of increasing the concentrations of bioactive peptides. Hence, it is imperative that the strain does not exhibit proteolytic properties, failing which the product will be destroyed. It is also important that the strain has the right specificity to produce a higher concentration of bioactive peptides. The concentration of ACE-inhibitory peptides seems to rely on a balance between the formation of bioactive peptides and its consequent breakdown into inactive peptides and amino acids, which in turn depend on storage time and conditions.

With the exception of *Lactobacillus delbrueckii* and *Lactobacillus lactis* which are used for milk fermentation, the uses of microbes as ACE inhibitor source have been less explored. Edible mushrooms *Tricholoma giganteum* have also been proved to have ACE inhibiting peptide. Many research groups have combed for ACE inhibitors in microbial sources such as *Doratomyces putredinis, Nocardia orientalis, Streptomycetes, Actinomycetes, Actinomadura, Spiculospora* and *Actinomadura* [9]. Based on these findings, this research focuses on isolating and identifying ACE inhibitor from the fermentation of beef extract using *Micrococcus luteus*.

MATERIALS AND METHODS

Microorganism and crude enzyme preparation

The isolated strain *Micrococcus luteus* (GenBank accession number Kf303592.1) was inoculated into a protease specific medium broth containing Beef extract (2.0 g/l), $MgSO_4.7H_2O$ (0.1482 g/l), KH_2PO_4 (0.3 g/l), $FeSO_4.7H_2O$ (0.003 g/l), Na_2HPO_4 (1.28 g/l), NaCl (0.05 g/l), NH_4Cl (0.1 g/l), Thiamine (0.03 g/l), $CaCl_2.2H_2O$ (0.0456 g/l). The inoculated broth was incubated at 37 °C for 1 d in an orbital shaker at 150 rpm. After fermentation, the entirely fermented broth was extracted, and the clear supernatant was recovered. The supernatant was filtered through a 0.45 mm cellulose acetate filter paper [10]. The crude enzyme extract was further subjected to the purification process. Before purifying the protein content [11], the ACE inhibitory activity of the crude extract was estimated.