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Original Article

ISOLATION OF ANGIOTENSIN-CONVERTING ENZYME INHIBITOR PRODUCING BACTERIA FROM COW MILK

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ABSTRACT

Objective: To evaluate the potential of protease producing organism for the production of Angiotensin I–converting enzyme (ACE) inhibitor by fermentation of various protein substrates.

Methods: Bacterial strains were isolated from cow milk collected in Coimbatore, Tamil Nadu, India by using serial dilution technique, plated on nutrient agar medium. The identity of the strain was ascertained by 16s rRNA gene sequencing method and was submitted to the NCBI GenBank nucleotide database. Various substrates were screened for ACE inhibitor production by the fermentation with the isolated strain.

Results: The isolated coded as BUCTL09, which showed a significant zone of clearance was selected and identified as *Micrococcus luteus* (KF303592.1). Among the seven substrates, only beef extract fermented broth showed an inhibition of 79% and was reported as the best substrate.

Conclusion: In the search for non-toxic, and economic ACE inhibitors as an alternative to the synthetic drugs, many natural ACE inhibitors have been isolated from a microbial source. In the present study, isolate BUCTL09 was selected for the production of ACE inhibitor from the beef extract. Findings from this study lead us to investigate this potent ACE inhibitor further for its biological properties and to explore the impending efficacy of the ACE inhibitor which may conceivably be developed into a prospective drug.

Keywords: *Micrococcus luteus*, ACE inhibitor, 16S rRNA gene sequence, Hippuric acid, Beef extract.

INTRODUCTION

Among the bioactive peptides, Angiotensin I–converting enzyme (ACE) inhibitory peptides derived from food proteins have attracted particular attention and have been studied comprehensively for their ability to prevent hypertension. These peptides could be used as a potent functional food additive and represent a healthier and natural alternative to ACE inhibitor drugs. Selecting the proper enzyme to hydrolyze the protein is a key factor in obtaining peptides that exhibit greater levels of ACE inhibitory behavior [1]. ACE is a dipeptide hydrolase that catalyzes both the formation of the potent vasoconstrictor, angiotensin-II (Ang-II), and the deactivation of bradykinin, a vasodilator peptide. Therefore, substances such as synthesized chemical drugs (e. g., captopril) or natural ACE inhibitory peptides can inhibit ACE activity and can cause a drop in blood pressure. These findings have been shown in both hypertensive human subjects and spontaneous hypertensive rats (SHR) [2].

Ang II is the principal biologically active peptide that causes arteriolar vasoconstriction and stimulates aldosterone secretion and thus plays an important role in hydromineral balance. Ang II has a potential role in various aspects of tumor progression and targeting Ang II production, or action could prove useful in anticancer therapy [3]. Researchers hope a better understanding of the angiogenesis process will help them in cancer treatment. There are many reports on ACE inhibitory peptides derived from food proteins, their physiological and pharmacological effects and their prospects for application in preventing hypertension and for therapeutic purposes. This study was intended to isolate protease producing organism for the production of ACE inhibitor by the fermentation of various proteinaceous substrates.

MATERIALS AND METHODS

Isolation of bacteria from milk

Raw unpasteurized milk samples of the cow were collected from the local area of Coimbatore, Tamil Nadu, India. The milk was collected in sterile screw cap tubes and processed within 3 h and used for further studies. Milk samples were serially diluted and were spread plated on nutrient agar medium. The plates were then incubated at 37 ºC for 48-72 h. Individual colonies with typical characteristics of being small (2-3 mm diameter) with entire margins were picked from each plate and transferred to nutrient agar medium. All isolates were initially examined by Gram's staining, cell morphology, and proteolytic properties.

Screening of protease producing bacteria

The isolates were screened for protease production by gelatin clear zone method using protease specific medium containing KH_2PO_4 (0.025 g/l), Yeast extract (0.25 g/l), Gelatin (2.5 g/l), NH_4HPO_4 (0.025 g/l), $CaCl_2$ (0.025 g/l), $MgSO_4.5H_2O$ (0.0125 g/l) and Agar (3.75 g/l). The diameters of clear zones were measured after 24 h of incubation at 37 °C by flooding the plates with mercuric chloride solution [4]. The isolate which produced the largest zone was selected for further study.

Identification of bacteria

Morphological and biochemical characterizations

Identification of bacteria was performed according to their morphological, cultural, physiological and biochemical characteristics [5]. Gram's staining, production of catalase, carbohydrate fermentation patterns, growth at 37 °C in nutrient agar medium, methyl red (MR) and Voges-Proskauer (VP) test in MR-VP medium and indole production in tryptone broth as described by Bergey's Manual of systematic Bacteriology were performed to identify the genus of bacteria.

Molecular characterization of bacteria

DNA isolation

Genomic DNA was isolated from the test organism [6]. Amplification of 16s rRNA gene was done with Universal primers: Forward primer 5'-GAGTTTGATCCTGGCTCAG-3'; Reverse primer 5'-ACGGCTACCT-TGTT