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Original Research Paper

Microbiology

SCREENING OF ASPARAGINASE ENZYME PRODUCING BACTERIA FROM RHIZOSPHERE SOIL OF UDHAGAMANDALAM REGION

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ABSTRACT

The study isolated microbes from rhizosphere soil using serial dilution techniques. The soil bacterial strains were screened for asparaginase activity using the Rapid Plate Assay method. The maximum asparaginase activity was observed in the SI 1 isolate, with a specific activity of 79.027 μmol/mg/min. The enzyme extract's antioxidant and free-radical scavenging activity were also determined. L-asparaginase is a crucial enzyme in the pharmaceutical and food industries, requiring specific properties for human safety and efficacy. It reduces acrylamide concentrations in food, maintaining nutritional and sensory properties.

KEYWORDS : Bacteria, Asparaginase, Rhizosphere, Enzyme

INTRODUCTION

Asparaginase is an enzyme that can be microbially produced. The L-Asparaginase has gained attention in recent years due to its significant applications in the food industry to prevent acrylamide formation. The enzyme asparaginase is added to certain foods. Under specific cooking conditions, asparagine can react with certain carbohydrates in the food to form acrylamide, a potential neurotoxin and human carcinogen. By reducing the amount of asparagine in food before cooking or processing, the amount of acrylamide that can be formed will also be reduced. In recent years, to reduce exposure to foodborne acrylamide, the food industry has been strongly encouraged to develop and implement acrylamide-reduction strategies. Asparaginase is a powerful tool for the food industry, and it is likely that its use will increase in the future. The occurrence of this enzyme has been reported in Bacteria, Fungi, Actinomycetes. Bacteria are an attractive microbial group which provides an effective source of L-Asparaginase enzyme.

Methodology

Soil samples were collected from the rhizosphere of the medicinal plants in Udhagamandalam, The Nilgiris District. Top layer of soil (about 1cm) was removed, and the soil samples were collected from a depth of 12-15 cm from the soil surface and stored in sterilized polyethylene ziplock covers, aseptically and transported to the laboratory.

The bacterial population present in the collected soil sample was isolated by carrying out the serial dilution technique. Accordingly, the soil suspension (1.0g soil in 10 ml of sterile distilled water) was serially diluted up to 10^{-8} dilution.0.1 ml of each dilution was uniformly spread on nutrient agar plates and incubated at 37^{0}C for 24-48 hours. The nutrient agar plates were incorporated with the antibiotic cycloheximide to prevent fungal growth. After the completion of the incubation period, the bacterial colonies were selected for the screening procedure for asparaginase enzyme. The selected colonies on the nutrient agar plates were inoculated over the Asparagine Dextrose Salt Agar (ADS) as simple streaks. The uninoculated medium with phenol red serves as a control plate. The colonies with pink zones were selected for the production of asparaginase enzyme.

The quantitative estimation of enzyme activity was done with the bacterial enzyme extracts. The crude enzyme extract obtained from the enzyme extraction step is used as the asparaginase enzyme sample. The rate of hydrolysis of L-Asparagine was determined by measuring the release of ammonia using Nessler's reaction. The reaction mixture contained 0.5 ml of enzyme sample, 0.5 ml of 0.05 M Tris-HCl buffer (pH 8.6), and 0.5 ml of 0.04 M L-Asparagine. The reaction mixture was incubated at 37^{0}C for 30 minutes. The enzyme activity was stopped after 30 minutes of incubation time by the addition of trichloroacetic acid (TCA 10% w/v). The mixture was then centrifuged at 10000 rpm for 5 minutes and 0.5 ml of the supernatant was taken and to it, 7 ml of distilled water was added; 1 ml of Nessler's reagent was added to the reaction tube and kept at 20^{0}C for 20 minutes. The control that contained only TCA without the enzyme was used as the control. The blank contains distilled water and the Nessler's reagent. The absorbance was measured at 450 nm using spectrophotometer. The amount of ammonia liberated was calculated using ammonium standard curve. One unit of L-Asparaginase activity is defined as release of one micromole of ammonia per hour at 37^{0}C and pH 8.6.

The antioxidant or free radical scavenging activity of the enzyme extracts was measured on the basis of a decrease in the absorbance of the ethanolic solution of stable DPPH. DPPH means 1,1-diphenyl-2-picrylhydrazyl free radical exhibit a dark purple color at absorbance 530 nm. After scavenging, a light yellow color was observed. The DPPH solution was made with methanol. About 0.5 ml of the sample was taken for the assay. To that, 3 ml of ethanol and 0.3 ml of DPPH radical solution were added. The mixture was jolted smartly and unbroken at room temperature for 30 minutes in the dark. Absorbance was measured. Scavenging activity was calculated

RESULT

About six bacterial isolates were obtained. These isolates were subjected to primary screening for asparaginase enzyme production. Among six soil isolates, three isolates produced pink zones. The three soil isolates were named as SI 1, SI 2 and SI 3.

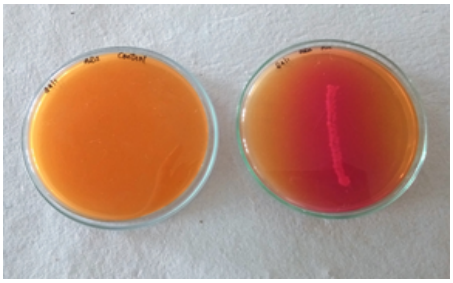

Figure 1: Isolate SI1 showing asparaginase activity in ADS medium

The quantitative estimation of asparaginase enzyme extracts was done by the Nesslerization method. The absorbance was measured at 450 nm using a spectrophotometer. The amount of ammonia liberated was calculated using the ammonium standard curve. One unit of L-Asparaginase activity is defined as the release of one micromole of ammonia per hour at 37^{0}C

and pH 8.6

Table1: Enzyme Activity Of The Asparaginase Enzymes Produced By The Isolates

SOIL ISOLATES	ABSORBANCE AT 450 nm	ENZYME ACTIVITY (IU/ml)
SI 1	1.05	37.14
SI 2	0.295	23.728
SI 3	1.56	25.42

Table 2: Antioxidant Activity Of The Asparaginase Enzyme Produced By The Isolate Si 1

SAMPLE	CONCENTRATION	ABSORBANCE AT 517 nm	ANTIOXIDANT ACTIVITY (%)
SI 1 (with maximum enzyme activity)	10	0.035	90.48
	20	0.061	83.42
	30	0.086	76.63
	40	0.119	67.66
	50	0.238	35.32
CONTROL	CONTROL	0.368	0

CONCLUSION

An intriguing enzyme with significant uses in the food and pharmaceutical industries is L-asparaginase. However, certain characteristics are necessary for its application in various industrial sectors to ensure human safety. In addition to fewer side effects, such immunological inactivation and hypersensitivity, an effective action is necessary as a chemotherapeutic drug. This enzyme preserves the nutritional value and sensual appeal of food by reducing the levels of acrylamide, a substance that can cause cancer in humans.

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