Short Communication

Extraction of Pterin deaminase from *Micrococcus species* isolated from soil

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Abstract:

Background: Pterin deaminase is an important enzyme which majorly involved in the biosynthesis of Pteridines. This enzyme possesses anticancer activity and present in different organism. The present study aimed to isolate the enzyme from *Micrococcus luteus.*

Methodology: The bacteria was isolated from the soil and cultivated in the modified media to synthesize pterin demainase. The extraction of intra cellular enzyme was carried out by sonication technique. The crude extract was purified using precipitation method.

Results: The enzyme activity and protein content prepared from the crude isolate were found to be 0.171 IU and 1.53 mg/mL respectively. After precipitation the specific enzyme activity was increased to 1.345IU/mg.

Conclusion: Further study has to be carried out to study the characterization of the Pterin deaminase enzyme.

Keywords: *Pterin deaminase, Micrococcus luteus, Precipitation method*

Introduction

Enzymes are mainly known for catalyst in speeding up the biochemical reactions in living organisms. But enzymes are having therapeutic properties with minimal toxicity. One such enzyme is Pterine deaminase possesses various biological functions including anti cancer activity (Thandeeswaran et al., 2019). Pterin deaminase belongs to hydrolyses enzyme family which mainly involves in the synthesis of pteridines. This enzyme acts mainly on the carbon nitrogen bonds rather than the peptide bonds which is the important property of this organism (Murugesan et al., 2017). In many organisms this enzyme is widely present as intracellular, extracellular or membrane bound enzyme. Microorganism exhibits the significant contribution in the health sector with various products to improve the well being of the people (Dhanapal et al., 2022). This enzyme was extracted from different microorganisms including *Saccharomyces cerevisiae* (Murugesan et al., 2017); *Agrobacterium tumefaciens* (Dhanapal et al., 2022); *Bacillus cereus* (Thandeeswaran et al., 2019). In the present study pterin deaminase enzyme was isolated from *Micrococcus luteus* a pigment producing microorganism and studied its biological properties.

Materials and Methods

Isolation of bacteria from soil

Micrococcus luteus was isolated from the soil and cultured in the nutrient agar

medium. Pure culture was obtained from the isolated cultures and stored at 4°C.

Pterin deaminase production from Micrococcus luteus

The fresh inoculum was prepared by taking the single colony of bacteria from the pure culture stock and inoculated in the nutrient broth. The media was incubated at 37°C for 24 hours at 125 rpm. Modified liquid medium was used to increase the enzyme production in microorganism.

Table 1: Composition of Modified liquid medium (g/L)

K_2HPO_4	-	1.5
KH_2PO_4	-	0.5
$MgSO_4.7H_2O$	-	0.2
Folic acid	-	3.16
Difco nutrient broth	-	5.0
Difco yeast extract	-	0.1
Dextrose solution	-	50 mL
pH	-	7.0

Composition of Difco nutrient broth (g/L)

Peptone	-	0.05
Beef extract	-	0.016
Sodium chloride	-	0.026

10% of the inoculum was added to the modified medium and incubated at 37°C, for 36 hours in the rotary shaker at 125 rpm.

Extraction of intra cellular enzymes:

After the incubation period, the media was centrifuged at 10,000 rpm for 30 minutes at 4°C. The cell pellet was harvested and washed thrice with autoclaved distilled water at cold conditions. To the washed pellets, Tris HCl buffer (50mM, pH 8.5) was added and sonicated for 3 minutes at the temperature of 25°C. After the sonication process, the cells were centrifuged at the rpm of 20,000g for 30 minutes at 25°C. The obtained supernatant was collected and this crude extract contain pterin deaminase enzyme which was confirmed by Mashburn and Wriston (1964).

Enzymatic assay to confirm the presence of Pterin deaminase:

To the 340 µl of the folic acid (10mM prepared in 50mM Tris-HCl buffer, pH 8.5), 40 µl of the Tris-HCl buffer (50 mM) was added and incubate for 300 seconds. To this mixture 50 µl of the crude enzyme mixture was added and incubated it for 10 minues. After the incubation period, 20 µl of Trichloro acetic acid was added to arrest the reaction. Centrifuge the tubes at the speed of 8000 RPM for 10 minutes. The supernatant was collected and made up to 500 µl with double distilled water and 500 µl of Nessler's reagent was added. After 10 minutes of incubation the absorbance was measured at 480 nm. Ammonium sulphate was used as standard.

Estimation of Protein

Protein estimation in the crude extract was carried out by Lowry's method.

Purification of Pterin deaminase by precipitation method

To 10 ml of the crude enzyme, 30 ml of ice cold ethanol was added and incubated at 4°C for 24 hours. The crude mixture was centrifuged at 9000 rpm for 20 min at 4°C. After discarding the supernatant, the pellet was mixed with equal volume of 50 mM Tris-HCl buffer, pH 8.5. After the precipitation process, the enzyme activity was measured.

Results and Discussion:

Pteridine is a heterocyclic ring fused ring compounds present have various biological functions. It plays an essential role in diverse group of organism. In butterfly it helps in development of butterfly wing colour. In humans it acts as an co-factor in the synthesis

of various amino acids. In the catabolic pathways of Pteridines, Pteridine deaminase was isolated which involved in the deamination reaction of folic acid to deaminofolic acid with the liberation of Ammonia (You et al., 2022). Pterin deaminase are classified in to the class of amidohydrolase enzyme involved majorly in the hydrolyzation of pteridines to liberate lumazine derivatives and ammonia. This enzyme was first reported in the bacteria *Alcaligenes metalcaligenes*[6]. In the fungi this enzyme was isolated *Aspergillus* spp and *Mucor*spp[7]. In mammalian models this enzyme was first reported in rat liver[8]. In the present study, Pteridine deaminase was isolated from *Micrococcus luteus.*

Table: 1. Pterin deaminase production in *Micrococcus luteus*

Pterin deaminase activity (IU)	Protein content (mg /mL)
0.171	1.53

Table 2. Purification profile of pterin deaminase from *Micrococcus luteus*

Purification step	Total Enzyme activity (IU)	Total Protein content (mg)	Specific activity (IU/mg)	Purification fold
Crude	7.15	153	0.112	1
Precipitation	11.7	8.7	1.345	12.01

The identified *Micrococcus luteus* was cultured in modified medium consisting of folic acid. The enzyme activity and protein content prepared from the crude isolate were found to be 0.171 IU and 1.53 mg/mL respectively (Table 1). Purification of crude enzyme is very essential to improve the specific activity of the enzyme[9]. Ethanol, the common alcohol widely used in the precipitation of enzymes and nucleic acid. Addition of ethanol enhances the conformational changes in the protein that helps to aggregate the protein by inter helical interaction[10]. In this study purification was carried out using precipitation method. After precipitation the specific enzyme activity was increased to 1.345IU/mg (Table 2). Previous study also showed the pterin deaminase enzyme activity was increased after ethanol precipitation.

Conclusion:

This study showed the presence of pterin deaminase in *Micrococcus luteus.* Further study has to be carried out to explore the biological property of the enzyme.

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