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# Enzyme Activity of Earthworm Coelomic Fluid in Leukoderma Peoples

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**ABSTRACT**

The amino acid profile of earthworms from Tamilnadu namely *Perionyx excavatus* were evaluated in this study. The amino acid analysis was conducted using Thin layer chromatography (TLC) .Two essential aminoacids namely Phenylalanine and Tyrosine two non-essential amino acids namely lycine and Proline were identified. Melanin gives colour to our skin , the enzyme tyrosinase is the responsible for the melanin formation. In this study found the aminoacid tyrosine.

**Key words**: Essential, Non essential, amino acids, *Perionyx excavatus*

## INTRODUCTION

Vermiculture is a new development in biotechnology based product. An earthworm is more familiarly known to the student of biology. In olden days earthworm were mainly used in the agricultural field, but now a days they are being used in the medical field, because of its amazing antimicrobial activity. Leukoderma means white patches on the skin Leuko means white, derma means skin, so white skin means leukoderma. Medically speaking, the melanin pigments gives colour to our skin. In India so far no work has been done in the Leukoderma recovery. In the present investigation an attempt has been made to investigate the earthworm coelomic fluid contain tyrosine which occurs in animals, plants and certain bacteria is due to the action of polyphenol oxidases (or) tyrosinases. Previously it was believed than an enzyme, dopa oxidase, catalyzes the formation of melanin from dopa, lerner considers that the enzyme tyrosinase is responsible for the melanin formation. The coelomic fluid have biologically active molecules and leukocytes which involve in the phagocytosis and encapsulation. It synthesizes and secretes the antibacterial molecules, cytotoxic proteins and enzymes.

### Collection of Coelomic fluid

The Earthworm (Mature) , *Perionyx excavatus* is collected and identify in the Molecular biology lab in Jamal Mohamed College, Tiruchrappalli, Tamilnadu ,India.

### Harvesting of Coelomocytes

Extrusion fluid preparation

- Phosphate buffer
- Ethylene diamine Tetra Acetic Acid (EDTA) (2.5g/l)

Equal quantity of phosphate buffer and EDTA solutions is add to make extrusion fluid.

### Methodology

The mature , *Perionyx excavatus* is immersed in clean , cool water for 5hr to eliminate gastro intestinal metabolites and contaminants. They are rinsed and rapidly dry on a filter paper and are subsequently excited with heat shock for 15 seconds,after the treatment the coelomic fluid release through their epidermal dorsal pores. After the centrifugation for 10 minutes at 4°C the cell free supernatant of coelomic fluid is collect store at -20°C.

**Determination of Coelomic fluid Tyrosine by Fluorimetric method**

Tyrosine (Udenfriend, 1962 )

The reaction of tyrosine with an α-nitrose-β-naphthol reagent also containing sodium nitrite and nitric acid is used. Excess of the reagent is removed with dicholoroethane.

**Reagents**

- α-nitrose-β-naphthol, 2g in ehanol, 95ml absolute ethanol made to a litre with water. Before use mix 2 volumes with 3 volumes of 3 mol/nitric acid (189 ml concentrated acid/ l) and 2 volumes of 10 mmol/ sodium nitrite solution (8.5g/l).
- Dichloroethane
- Trichloroacetic acid 600mmol/l (98g/l)
- Tyrosine standards, 150, 300, 600µl/l (2.71, 5.43 and 10.8mg/100ml)in water keep at 4°C.

The 100ml of coelomic fluid (the test ), 100ml standard (or) 100ml of water (the blank), and 100ml trichloracetic acid to stand 10 minutes and centrifuge add 500ml nitrisonaphthol reagent to 50ml supernatant incubate at 33°C for 20 minutes then add 2.5ml water and 7.5ml dichloroethane to each, mix and centrifuge. Transfer the aqueous upper layer to other test tubes and allow to stand for about 40min at about 25°C. Read the fluorescence within 30 minutes at 570nm with an activating wavelength of 460nm.

**Calculation**

$$\text{Coelomic fluid tyrosine}(\mu\text{mol/l}) = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \text{ x 150,300 or 600 or x 2.71, 5.43or 10.8 mg/100ml.}$$

**Protein Estimation–Bradford Method**

Protein reacts with Bradford reagent to give coloured complex (Blue). The colour is formed due to the reaction of coomassie brilliant blue (G 250), ethanol and phosphoric acid in the presence of protein. The intensity of colour depends on the amounts of these aromatic amino acids present and will thus vary from different protein (Bradford, 1976).

**Procedure**

Total protein content in the sample is estimate using Bradford method. One milliliter of each sample is taken and mix with 2.5ml of Bradford reagent. The test tubes are left for 20 minutes until the appearance of blue colour, is measure at 595nm in UV visible spectrophiotometer (Ferkin Elmer, GERmany ). A blank solution containing 1ml of the distilled water and 2.5ml of Bradford reagent is also prepare. The bovine srum albumin (BSA) is use as standard. The protein concentration is express in mg/ml of sample.

**Amino acid separation by thin layer chromatography**

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Mixed 20g of silica gel G with 30ml of water and stirred well until fine slurry without clumps obtained. A mixture of Butanol : Acetic acid: water in the ratio of 4:1:5 was used as a solvent (mobile phase)The mobile phase was allowed to migrate past toward the far end of the plate during the mixture was separated into various components. The irrigation solvent to run a distance of 12cm from the spot.

Remove the plates from the tank and immediately dried at 60°C for 30 minutes. The separated amino acids spots were detected by spraying the plates with ninhydrin. The ninhydrin sprayed on plates and heated at 110°C. The compounds become and could be seen as brown spots.

The distance traveled by each compound from the origin relative to the solvent front is defined as the Rf. The Rf value for the amino acid is calculated.

$$\text{Rf} = \frac{\text{Distance traveled by the substance from the origin}}{\text{Distance traveled by the solvent from the origin}}$$

## RESULTS AND DISCUSSION

Two essential aminoacids namely Phenylalanine and Tyrosine two non-essential amino acids namely lysine and Proline were recorded in this study through the Thin layer chromatography (Table 1). The range of essential aminoacids obtained from the *Perionyx excavatus* , the same range for different fishmeal brands by Bonar (1993) .(Table 2).

**Table 1: To identify the Aminoacid fraction in the Coelomic fluid**

| S. No | Amino acid fraction | Distance moved by solvent (A) | Distance moved by solute (B) | Rf value= B/A |
|---|---|---|---|---|
| 1 | 1 | 10 | 1. 8 | 0.18 |
| 2 | 11 | 10 | 4.1 | 0.41 |
| 3 | 111 | 10 | 6.9 | 0.69 |
| 4 | 1V | 10 | 9.2 | 0.92 |

Rf value of Aminoacid 1 = 0.18-(Lysine)
Rf value of Aminoacid 11 = 0.41-(Proline)
Rf value of Aminoacid 111 =0.69-(Phenylalanine)
Rf value of Aminoacid 1V = 0.92-(Tyrosine)

**Table 2: The Concentration of aminoacid in Coelomic fluid compared with Fishmeal brands**

| Essential Aminoacid | Fishmeal brand(72%) (Baonr,1993) | Earthworm fluid (This study) |
|---|---|---|
| Lysine | 5.52 | 5.58 |
| Proline | 2.25 | 3.23 |
| Phenylalanine | 2.71 | 3.52 |
| Tyrosine | 3.30 | 3.75* |

The amino acid profile obtained in this study is comparable to that of fishmeal brands in the study of Boanr (1993) and it revealed that the aminoacid profile of these earthworm species to compare with fishmeal. Guererro (1981) working on *Perionyx excavatus* showed that the worm is a good source of protein. Stafford and Tacon (1984) showed that *Dendrodrilus subrubicundus* contains 65% crude protein. The study of Edwards and Niederer (1988) also revealed that earthworms are an excellent source of protein. The study of Vielma-Rondon (2003) also showed that earthworms have been shown contain 60-70% crude protein .The value of essential amino acids concentration obtained in this study were on the higher side of the range and this agrees with the study of Edwards(1985) which showed that earthworm protein contain a higher content of essential aminoacids such as lysine and methionine than either meat or fishmeal. Dynes (2003) also confirmed that the aminoacid concentrations of earthworm such as *Eisenia fetida, Lumbricus terrestris, Perionyx excavatus* were similar to that of fishmeal or even better, as the case is in this study. This suggests that earthworm species from Nigeria has comparable amino acid profile and concentration with their counterparts from the temperate region. As such these earthworm species could therefore be incorporated as dietary supplement to supply such limiting aminoacids as lysine and methionine in the diets of fish or other animals. In this present investigation to identify the aminoacid Tyrosine.

## CONCLUSION

Coelomic fluid have also proven to be useful in improving the skin appearance of patients suffering from leucoderma. Use of this fluid can help to compat leucoderma effectively and to get rid of those white patches. The depigmented patches fade away with regular application of coelomic fluid. Being free from harsh chemicals, this fluid does not produce any side effects, such as skin irritation. This skin disorder responds quite well to this fluid.

To apply the fluid everyday and will notice the considerable improvement within 2 months. The fluid has to be applied twice a day the fluid completely absorbed in the affected area.

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