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Isolation and screening of pterin deaminase producing fungi from soil samples

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Abstract
Pterin deaminase is a folate deaminating enzyme has been reported to have antitumour activity against leukemic cell line and melanoma induced in mice. In the present study, a total of twenty five fungal cultures were isolated from soil sample collected in and around the city of Coimbatore district. The diversity of fungal isolates was found to be higher in the cultivated land soil compared to unattended barren land and coconut plantation soils. A rapid plate assay method was used for the first time to screen the pterin deaminase producing fungus using folate as a substrate in modified czapek Dox agar medium. The strain CLS-6 was selected based on the zone of clearance and pink colouration around the zone with a maximum enzyme activity of 40.2 IU/ml and 36.5 mg/ml of protein for further purification and characterization.

Keywords: Pterin deaminase, Lumazine, Folate, Substrate, Cancer

1. Introduction
Fighting cancer is considered as one of the most important areas of research in medicine and immunology. Folate-degrading micro-organisms which may be used as source of enzymes in the treatment of malignant tumours. Folic acid is an essential vitamin for nutritional requirements of both normal and cancer cells. One of the such enzyme Pterin deaminase is belongs to enzyme class hydrolase which is a water soluble [5]. The enzyme deamination the substrate folic acid to products lumazine and ammonia. Pterin deaminase is reported in various organisms such as *Aspergillus*Y8-5 [4], *Bacillus megaterium* [12], *Bacillus subtilis* [13], *Alcaligenes faecalis* [8], *Rhizopus arrhizus* IAM 6052 and *Mucor lam prosporus* IAM 6114 [5]. Microbes are better source for the production of pterin deaminase because they can easily cultured, extraction and purification as well as the methods of this process from them is also convenient. The antineoplastic activity results from depletion of the folic acid by pterin deaminase. The enzyme converts the folic acid to the product lumazine, further it cannot be utilized for DNA synthesis. There is less chance of resistance against cancer cells due to the attack of the enzyme on folic acid and upregulation folate dependent enzymes. Earlier no screening method available for isolating pterin deaminase, a modified technique used for the isolation of antitumour enzyme L asparaginase [1] and isolation of industrially important enzyme glutaminase [3] was adopted in the present investigation. Taking into consideration of fungal enzymes, the present research was initiated to isolate, and screen the fungal strains for novel therapeutic enzyme pterin deaminase.

2. Materials and Methods
2.1 Collection of soil samples
A total of 26 soil samples to a depth of 6-10 cm deep pits [10] from seven sites of coconut plantation soil (CPS), eight sites in cultivated land oil (CLS), six sites in compost soil (CS) and five sites in barren land soil were collected in and around the Coimbatore regions, India. The samples were placed into a sterile polythene bags and carried to department for further microbial analysis.

2.2 Isolation of fungi
Soil samples (1g) were serially diluted with sterile water and plated in pour plate technique on Sabourauds dextrose agar medium composed the plates were incubated at 28 °C for 7-14

days and pure culture was obtained by quadrant streaking method. The fungi were isolated by soil dilution plate method and preserved at 4 °C for further characterisation [11].

2.3 Screening of pterin deaminase producing fungi

The screening was done using the rapid plate assay technique. The methodology was modified based on Gulati *et al.* [1]. Modified czapek Dox medium containing folic acid, KH2 PO4, KCl, MgSO4.7H2O, Zn SO4.7H2O, FeSO4.7H2O, phenol red at the pH of 6.2 and was supplemented with 0.009% (v/v) phenol red as indicator. Control plates were MCD medium without dye and folic acid. Then, the petriplates were inoculated with the 25 selected fungal isolates and incubated at 30 ºc for 48 hrs. The isolates that showed pink colour zones around the colonies indicated pterin deaminase production and were selected for determination of enzyme activity.

2.4 Production of pterin deaminase

Pterin deaminase production by fungal isolate was carried out by submerged fermentation condition. A 100 ml of sterile medium was used for production. The medium was inoculated with the culture. The flask was incubated in an orbital shaker at 120 rpm at 37 C for 24 hrs. The fungal cell mass was separated by centrifuged at 8000 rpm for 10 min at 4 C. The supernatant was used as crude enzyme source to know the enzyme activity and protein assay.

2.5 Pterin deaminase assay

The pterin deaminase enzyme activity of culture filtrates was determined by Mashburn and Wriston method [7]. In this method, 340 µl of folic acid (0.5M), 40 µl (50 mm) of Tris-HCl buffer (pH 8.6) were added and kept for 5 min incubation. Then 50 µl of enzyme was added and incubated for 10 min and the reaction was arrested using 20µl trichloro acetic acid. The mixture was then centrifuged for 5 min at 8000 rpm. A blank with boiled enzyme was set. The supernatant, made up to 500 µl of distilled water and added 500 µl of Nesslers reagent and incubated for 10 min. The ammonia released was spectrophotometrically estimated at absorbance 480 nm. Ammonia standard was also simultaneously run using ammonium sulphate.

2.6 Estimation of protein

Estimation of protein was determined by using Lowery *et al.* [6] method. The standard stock bovine serum albumin (BSA), at a concentration of 1000 µg/ml was prepared. A working standard solution, 0.2 to1 ml at a concentration of 100 µg/ml was taken in a test tube. Then it was made upto1ml using distilled water to give concentrations ranging from 20 to100 µg/ml. 1 ml of folins cocatteau reagent was added to each test tube. After 30 min of incubation, the absorbance was measured at 660 nm using UV-VIS spec and the protein content estimated.

3. Results

A total number of 25 fungal cultures were isolated for pterin deaminase production from four different soil samples (Table 1) using sabourauds dextrose agar medium at 37 °C for 14 days. Among the isolates, eighteen positive potential strains showed different range of zone of diameter (Table 2) in modified czapek Dox agar. In these positive strains, eight isolates were selected based on the diameter of coloured zone > 0.5 cm with pink zone of clearance. In this study, strain CLS-6 (Fig.1) from cultivable land soil exhibited the highest zone of diameter (2.8 cm). The organism CLS-6 showed pink zone around the colonies on modified czapek Dox agar containing phenol red as an indicator which increase in pH due to ammonia accumulation in the medium. The dye indicator was yellow in colour at acidic condition and turned to pink at alkaline condition (Fig.2). Further these isolates were subjected to the secondary screening for enzyme activity by Mashburn and Wriston (7) method**.** The fungal isolate CLS-6 showed maximum activity of 40.2 IU/ml and 36.5 mg/ml of protein (Fig. 3).

Table 1: Soil fungal isolates from different ecosystem type

S. No	Ecosystem type	No. of soil samples collected	No. of colonies isolated
1	Coconut plantation soil (CPS)	7	3
2	Cultivated land soil (CLS)	8	11
3	Compost soil (CS)	6	6
4	Barren land soil (BLS)	5	5
	Total	26	25

Table 2: Screening of the fungal strains for Pterin deaminase production on modified Czapek Dox Agar

S. No.	Fungal isolates from different sources	Diameter of zone (cm)	Zone of clearance
1	CPS 1	0.1	+
2	CPS 2	0.4	+
3	CPS 3	-	-
4	CLS 1	-	-
5	CLS 2	-	-
6	CLS 3	2.2	++
7	CLS 4	0.1	+
8	CLS 5	0.2	+
9	CLS 6	2.8	+++
10	CLS 7	1.5	++
11	CLS 8	-	-
12	CLS 9	1.2	++
13	CLS 10	1.8	++
14	CLS 11	0.5	++
15	CS 1	0.1	+
16	CS 2	0.3	+
17	CS 3	1.33	++
18	CS 4	0.98	++
19	CS 5	-	-
20	CS 6	0.3	+
21	BLS 1	-	-
22	BLS 2	-	-
23	BLS 3	0.3	+
24	BLS 4	0.6	+
25	BLS 5	0.4	+

+++-very good ++ good + Average - No zone

Strain (CLS-6)

Fig 1: Selected CLS-6 Strain on SDA agar plates

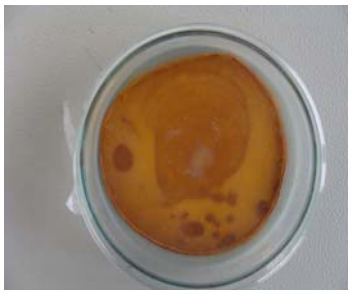

Fig 2: Selected CLS -6 strain on Modified czapek Dox agar

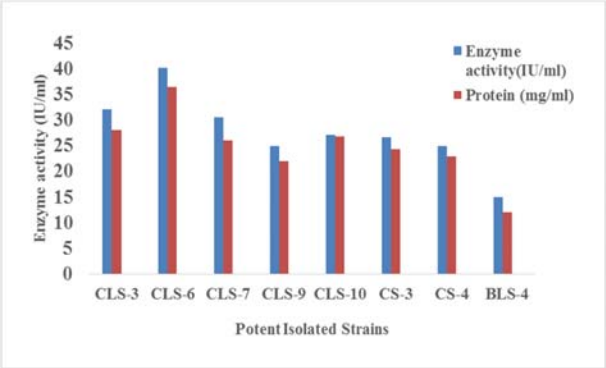

Fig 3: Potent isolated strains showing Pterin deaminase activity

4. Discussion

In the present study**,** the diversity of fungi isolates was found to be higher in the cultivated land soil compared to unattended barren land and Coconut plantation soils. This indicates the superior quality of soil that can be utilized for cultivation. According to Wahegaonkar *et al.* [14], maximum 85 fungal species from 45 genera were isolated in agricultural soils. It has been found that more number of genera and species of fungi exist in soil than in any other environment [9]. Among the various genera of fungi in the four ecosystem types studied by Wahegaonkar *et al.* [14], *Aspergillus* was the only genus that was distributed in all the types, indicating that it adapts easily to different environment well.

Folic acid as substrate for pterin deaminase was used as sole nitrogen and carbon sources to screen fungi for the production of enzyme. The basic principle is that enzyme catalyzes folic acid present in the medium producing ammonia, which gives raise to change in medium from neutral to alkaline. Twenty five isolates obtained from different soil samples after serial dilution method were subjected to screening based on the zone of clearance method [1]. The results revealed that out of 25 isolates from different sites of soil samples, the eighteen positive potential strains showed different range of zone of diameter in modified czapek Dox agar. The selected isolate CLS-6 (Fig.2) from cultivable land soil exhibited the highest zone of diameter (2.8 cm) and was maintained in SDA agar plates. The strain exhibiting zone of diameter above 0.9cm was referred to as good for pterin deaminase producers, those strains with zone diameter of 0.6 - 0.9 cm and those having below 0.6cm zone of diameter may be referred to as moderate and poor pterin deaminase producers respectively. The above strains exhibiting zone of diameter was classified based on L-asparaginase producing fungi [2]. In the present study the pink zone formation was observed around the colonies in presence of phenol red as an indicator which increase the pH and changed the colour of the medium. Further these isolates were subjected to the secondary screening, isolate CLS-6 exhibited maximum enzyme activity of 40.2 IU/ml and 36.5 mg/ml of protein (Fig 3). Other fungal isolates were showed low enzyme activity.

5. Conclusion

From this work, it was clearly showed that the cultivated land soil highly rich pterin deaminase producing organisms when compared to other type of soils. In almost all eight potent isolated showed nearest enzyme activity. However, in future the strain CLS-6 fungi will be taken to categorize its identification and purification of the enzyme.

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7. References

1. Gulati R, Saxena RK, Gupta R. A rapid plate assay for screening L-asparginase producing microorganisms. Lett. Appl. Microbiol. 1997; 24:23-26.
2. Hosamani R, Kaliwal BB. Isolation, Molecular identification and Optimization of Fermentation parameters for the production of L-asparaginase, an anticancer agent by *Fusarium equiseti*. International Journal of Microbiology Research. 2011; 3(2):108-119.
3. Imada A, Igarasi S, Nakahama K, Isono M. Asparaginase and glutaminase activities of microorganisms. Journal of General microbiology. 1973; 76:85- 99.
4. Kusakabe H, Kodama K, Machida H, Kuninaka A. Antitumour activity of pterin deaminase. Agric Biol. Chem. 1979; 43:1983-1984.
5. Kusakabe H, Kodama K, Midorika Y, Machida H, Kuninaka A, Yoshino H. Inhibition of growth of L5178Y Leukemia cells by a fungal folate-deaminating enzyme. Agric. Biol. Chem. 1974; 38:1753-1754.
6. Lowry OH, Rosenbrough NJ, Faer AL, Randall RJ. Protein measurement with the folin-Phenol reagent. J Biol Chem. 1951; 48:17-25.
7. Mashburn LT, Wriston JC. Tumour inhibitory effect of L-asparaginase from *E. coli*. Arch. Biochem. Biophys. 1964; 105:450-452.
8. Nutt Mc WS. Enzymic deamination and amide cleavage of folic acid. Arch Biochem. Biophys. 1963; 101:1-6.
9. Nagmani A, Kunwar IK, Manoharachary C. Handbook of Soil Fungi. Published by I. K. International Pvt, Ltd. New Delhi, 2005.
10. Pulikesi M, Baskaralingam P, Elango D, Rayuduc VN, Ramamurthy V, Sivanesan S. Air quality monitoring in chennai, India, in the summer of 2005. J Hazrdous. Material. 2007; 136:589-596.
11. Takahashi T, Jacqueline A, Lucas C, Esther MF. Occurrence and structural diversity of fungal metabolites with antibiotic activity. Quimica Nova. 2008; 31(7):1807-1818.
12. Takikawa S, Yokokawa CK, Tsusue M. Pterin deaminase from *Bacillus megaterium* – purification properties J Biochem. 1979; 85:785-790.
13. Tsusue M, Takikawa S, Yokokawa CK. The bacterial catabolism of pteridines. Dev. Biochem 1978; 4:153-158.
14. Wahegaonkar N, Salunkhe SM, Palsingankar PL, Shinde SY. Diversity of fungi from soils of Aurangabad, M.S., India. Annals of Biological Research. 2011; 2(2):198-205.