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Industrial important microfungal amylase enzyme optimization from microfungal diversity in wood debris soil in Sivakasi, Tamil nadu

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ABSTRACT

The seven micro-fungal species were isolated from wood debris soil by using Potato dextrose agar medium (PDA). The various pH, Carbone, nitrogen source conditions were optimized for amylase production. The present studies clearly indicates, the high level production of amylase around at pH 6.5 to 7.5 *Aspergillus niger* (375U/ml) followed by *Aspergillus ochraceus* (350U/ml), *Aspergillus terreus* (340 U/ml).

Keywords: Microfungal diversity - Industrial enzymes –Microfungal Amylase – Optimization

INTRODUCTION

Plant litter, agricultural recycling, and wood decomposition is an ecosystem process nearly on par in importance with primary production by vascular plants. An array of organisms differing in biological organization and functional capacities mediate the process. They range from small detritivorous invertebrates to bacteria and fungi. This variety suggests a large scope for mutualistic and competitive interactions among decomposers and consequent biodiversity effects on litter decomposition and agricultural litter recycling. Such effects may be further reinforced by the diversity of plant litter found under mixed vegetation. Their grand species composition and ability to produce a variety of enzymes and establish simple to complex ecological association with plants and other organisms.

Enzymes are among the most important products obtained for human needs through microbial sources. A large number of industrial processes in the areas of industrial, environmental and food biotechnology utilize enzymes at some stage or other. Current developments in biotechnology are yielding new applications for enzymes[13]. The role of many enzymes has been known for a long time. Their existence was associated with the history of ancient Greece, where they were using enzymes from microorganisms in bakery, brewing, alcohol production, cheese making[7]. The Selection of the right organism plays a key role in high yield of desirable enzymes. Fungi are microorganisms which are well known for their wide range of

novelty of enzymes they produce and enzymes of fungal origin are used in the industrial process for which, amount to billions of dollars of revenue annually[2]. Due to their diversity, fungi have been recognized as a source of new enzymes with useful and novel characteristics[3].

Nowadays enzymes are used in large scale textile industry such as amylase, cellulase, lipase and protease, lipase. The major classes of enzyme offering immediate application are the hydrolytic enzymes[9]. Among all industrial enzymes, hydrolytic enzymes account for 85%. Microbial enzymes are preferred to those from plants and animal sources because they are cheaper to produce and their enzyme contents are more predictable, controllable and reliable[12] and also because of their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation.

MATERIALS AND METHODS

Sample collection and isolation of fungi
Sampling site

The soil sample was collected from the agriculture field, wood debris, leaf litter in the area of sivakasi, virudhunagar district. The sample collecting site has located in the region of sivakasi is located in the Bypass road of long trees sub locality, sivakasi locality, virudhunagar district, Tamil nadu state.

The leaf litter soil was collected in the area of Bypass road of long tree is situated latitude is 9”45’ and longitude is 77”79’. The agricultural soil samples were collected in the area of Katalipatti sivakasi. Katalipatti is located in kaliappanagar sub-locality, sivakasi locality, virudhunagar district, tamilnadu state 626123. The latitude of katalipatti area is (9”, 45’) and longitude of katalipatti (77”, 78’).

The wood debris soil samples were collected in the area of Alangulam area, sivakasi. The Alangulam area is located in sithurajapuram sub locality, sivakasi locality, virudhunagar district, tamilnadu 6126123. The latitude of alangulam area, 9”.43’ and longitude of Alangulam area is 77”, 79’.

Wood debris soil

The wood debris soil was collected with the help of sterile spatuchela and transferred to sterile polythene zip cover for laboratory for fungal analysis.

Fungal isolation from soil samples

One gram soil was mixed thoroughly in 10ml of sterile water in a glass tube and shaken thoroughly. From this initial suspension, serial dilutions were prepared. One ml of the required dilution (1/1000) was pipetted into five replicate plates containing potato dextrose agar medium with antibiotic. The plates were incubated at room temperature in glass chambers under aseptic conditions for 4 days and then examined for fungal growth. All fungal colonies developed were recorded.

Slide preparation and identification

Lactophenol and Lactophenol cotton blue stain (Hi Media laboratories private limited) were used as the staining solution. Slides prepared were sealed with DPX mountant. Identification of the fungal species was done using Compendium of soil fungi[6].

Production medium for amylase

To prepare the production medium for the amylase using following components Starch-1g, Yeast extract-1g Dextrose -1g, in 250ml Erlenmeyer flasks. The effect of various carbon sources like glucose, sucrose, lactose, maltose have also been used for estimate the amylase production. The effect of different nitrogen source like ammonium sulphate, ammonium nitrate, potassium nitrate, casein used for estimate the amylase production. Each flask was adjusted to different pH such as 6, 6.5, 7, 7.5 and 8 using 0.1 N NaoH and 0.1 N Hcl. Pour 10ml of sterile distilled water on the slant containing fungal spores. Scrape with a wire loop of loosen the spores. Inoculate fermentation medium with 0.5ml spore suspension of fungi. The culture flasks were incubated in 28°c for 3 days[4].

Quantitative assay of amylase

The assay system consists of the following ingredients taken in the test tube 0.5ml of 1% starch in 0.1M phosphate buffer (pH 6.5) and 0.5ml of enzyme were incubated for 30 min at room temperature. The reaction was arrested by adding 1.0 ml of Dinitrosalicylic acid reagent and kept on boiling water bath for 5 min and 1.0 ml of distilled water was added. Blank was the same as above without incubation. Absorbance was measured at 540 nm against blank. Amylase activity can be found out with the help of glucose standard graph. Enzyme activity was expressed in units[4].

RESULTS

Quantitative assay of fungal enzymes at different pH

The seven fungal species such as *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Aspergillus terreus, Penicillium citrinum, Fusarium oxysporum, Curvularia lunata* were isolated from agricultural soil, leaf litter soil, wood debris soil by using Potato dextrose agar medium (PDA). The various pH, Carbone, nitrogen source conditions were optimized for amylase production. The maximum amylase activity was recorded in *Aspergillus Niger* (375U/ml) followed by *Aspergillus ochraceus* (350 U/ml), *Aspergillus terreus* (340 U/ml), *Aspergillus flavus* (330 U/ml), *Penicillium citrinum* (185 U/ml), *Curvularia lunata* (35 U/ml) and *Fusarium oxysporum* (25 U/ml) at pH 6.5 (Table-1). Similarly maximum cellulase activity was observed in *Aspergillus ochraceus* (365 U/ml), followed by *Aspergillus terreus* (355 U/ml), *Aspergillus flavus* (350 U/ml), *Aspergillus niger* (315 U/ml), *Fusarium oxysporum* (305 U/ml), *Curvularia lunata* (280 U/ml), and *Penicillium citrinum* (65 U/ml) at pH 7.5 (Table -1).

Table: 1. Micro-fungal Amylase activity in different pH

S.No	Fungal Species	Amylase activity in different pH (U/ml)				
		6	6.5	7	7.5	8
1	*Aspergillus niger*	85	375	330	270	105
2	*Aspergillus flavus*	95	330	350	240	230
3	*Aspergillus ochraceus*	75	350	335	135	250
4	*Aspergillus terreus*	120	340	300	240	195
5	*Penicillium citrinum*	65	185	205	200	160
6	*Curvularia lunata*	45	35	60	40	50
7	*Fusarium oxysporum*	30	25	35	20	15

Effect of Different carbon source on Micro-fungal amylase activity

The maximum amylase activity was recorded in *Aspergillus ochraceus* (210 U/ml) using sucrose followed by *Curvularia lunata* (205 U/ml) and *Fusarium oxysporum* (165 U/ml) using maltose as carbon sources. In *Aspergillus flavus* (190 U/ml), *Aspergillus ochraceus* (125 U/ml), *Aspergillus niger* (185 U/ml) *Penicillium citrinum* (100 U/ml) *Aspergillus terreus* (120 U/ml) using sucrose (Table-2).

Table: 2. Micro-fungal Amylase activity in different carbon sources

S.No	Fungal Species	Amylase activity in different carbon sources (U/ml)			
		Glucose	Maltose	Sucrose	Lactose
1	*Aspergillus niger*	10	20	185	125
2	*Aspergillus flavus*	25	40	190	110
3	*Aspergillus ochraceus*	35	125	210	100
4	*Aspergillus terreus*	25	20	120	65
5	*Penicillium citrinum*	45	10	100	70
6	*Fusarium oxysporum*	45	165	55	70
7	*Curvularia lunata*	85	205	80	105

Effect of nitrogen source on Micro-fungal amylase activity

The maximum amylase activity was recorded in *Aspergillus ochraceus* (355 U/ml), followed by *Aspergillus niger* (300 U/ml), *Curvularia lunata* (270 U/ml), *Penicillium citrinum, Fusarium oxysporum* (250 U/ml), *Aspergillus flavus* (220 U/ml), and *Aspergillus terreus* (210 U/ml) using potassium nitrate (Table-3).

Table: 3. Micro-fungal Amylase activity in different nitrogen sources

S. No	Fungal Species	Amylase activity in different nitrogen sources U/ml			
		Ammonium Sulphate	Casein	Ammonium Nitrate	Potassium Nitrate
1	*Aspergillus niger*	200	105	25	300
2	*Aspergillus flavus*	270	105	275	220
3	*Aspergillus ochraceus*	160	125	40	355
4	*Aspergillus terreus*	245	100	75	210
5	*Penicillium citrinum*	205	185	20	250
6	*Fusarium oxysporum*	210	165	160	250
7	*Curvularia lunata*	200	225	10	270

DISCUSSION

Among all industrial enzymes, hydrolytic enzymes account for 85%. Microbial enzymes are preferred to those from plants and animal sources because they are cheaper to produce and their enzyme contents are more predictable, controllable and reliable [12] and also because of their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation. Today, the new potential of using microorganisms as biotechnological sources of industrially relevant enzymes have stimulated renewed interest in the exploration of extracellular enzymatic activity in several microorganisms[5]. In industrial biotechnology, more than 30 different types of fungal enzymes are used commercially; e.g., α-amylase from *Aspergillus niger*, *A. oryzae*; cellulase from *Humicola insolens*, *Penicillium sunicalo*; glucoamylases from *A. phoenicis*, *Rhizopus delemar*; glucose oxidase from *A. niger*; laccase from *Coriolus versicolar*; pectinase from *A. niger, A. oryzae* and protease from *A. Melleus*[11]. An ideal industrial enzyme should possess high stability and high activity over a wide range of reaction conditions. Therefore an attempt has been made to study the diversity of micro-fungal species and discussing them in light of available information on diversity with respect to the ecosystem they occur in with an aim to understand their role, function, and application potential. Among the four fungal enzymes studied, amylase activity was recorded to be maximum in all the species.

Kathiresan and manivannan [8] also recorded good amylase activity in *Penicillium fellutanum* and *Aspergillus flavus*. Similarly in present studies the maximum amylase activity was recorded in *Fusarium oxysporum* (400 U/ml) followed by *Aspergillus flavus* (375 U/ml) *Curvularia lunata* (240 U/ml), *Aspergillus terreus* (210 U/ml) and *Penicillium citrinum* (164.5 U/ml).

The combination of maltose and soluble starch was selected as the best carbon source for maximal α-amylase production (8.6 U/mL) by *A. niger* isolate followed by soluble starch alone (7.7 U/mL), maltose (5.4 U/mL) and the others. Glucose greatly repressed enzyme synthesis and catabolic repression in α- amylase activity. Mixed substrates of lactose and maltose have also been reported for α-amylase production [16]. In present study maximum amylase activity was recorded in *Aspergillus ochraceus* (210 U/ml) followed by *Aspergillus flavus* (190 U/ml), *Aspergillus niger* (185 U/ml) using sucrose. Previous findings have shown that peptone, sodium nitrate and casein hydrolysate are good nitrogen supplements for amylase production in *A.fumigatus*, *A.niger*, *A.oryzae* [13, 14]. Similarly the maximum amylase activity of nitrogen source was recorded in *Aspergillus ochraceus* (355 U/ml), followed by *Aspergillus niger* (300 U/ml), *Curvularia lunata* (270 U/ml) using potassium nitrate.

CONCLUSION

The role of decomposition of organic matter is determined by the fungi depend on the ability to utilize the plant substrate. Besides the chemical composition such as Carbone and nitrogen and pH, environmental factors such as temperature, moisture content, availability of nutrients and energy-source, regulate the process of micro-fungal enzyme production. The fungal enzyme main key role for degradation for litter and soil component. The present studies clearly indicate, the high level production of amylase production optimization. The potential of using micro-fungal as biotechnological sources of industrially relevant enzymes have stimulated renewed interest in the exploration of extracellular amylase enzymatic activity in several fungal species.

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