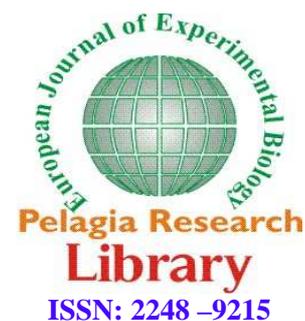




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Studies on amylase and cellulase enzyme activity of the fungal organisms causing spoilage in tomato

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ABSTRACT

Tomato, one of the most important vegetable in many countries has a worldwide economic and nutritive importance. It contains an energy value of 20 kcal per 100 grams of edible product. Quality of tomato fruits could be reduced by various diseases in the field. Fungi are responsible for spoilage of the fruits during storage. Fungi are very good sources of producing diverse enzymes that can degrade natural polymeric compounds such as cellulose, pectin and starch. The present study was under taken to isolate an efficient enzyme producing microorganisms from the spoiled tomato. Three different isolates were isolated from the spoiled tomato and screened for enzyme activity. In submerged fermentation the production of amylase by *Aspergillus* sp reached maximum 48U/ml at 72 hrs of incubation period. The production of cellulase by *Trichoderma* sp reached its maximum of 42 U/ml at 72 hrs. In solid state fermentation the production of amylase by *Aspergillus* sp reached maximum 108U/ml at 72 hrs of incubation period. The production of cellulase by *Trichoderma* sp reached its maximum of 56 U/ml at 72 hrs. These enzymes have commercial application in various industries such as starch, detergents, food, textiles, animal feed, pulp and paper, leather, chemical and biomedical products.

Keywords: *Aspergillus*, *Trichoderma*, Amylase, Cellulase, Fermentation.

INTRODUCTION

Tomato is a prevalent vegetable use both in raw form as salad, for garnishing various food items and added for taste in various cooked items. Tomato is also an important food component since it contains β - carotene, which is the precursor for vitamin A synthesis, extremely synthesis for vision. This increases the priority of safeguard its productivity and prevention from microbial spoilage. Tomato is highly prone to the spoilage of fungi especially *Aspergillus* species, *Penicillium* species and *Trichoderma* species [4]. Fungal phytopathogens are cause of many

plant diseases and much loss of crop yields, especially in tropical and subtropical regions [3,5]. The spoilage by these fungal species can be very serious and detrimental due to their ability to produce mycotoxins. These mycotoxins are not only limited to their area of infection by the fungus as these fruits and vegetables contain more of fluid and thus the mycotoxins diffuse rapidly throughout the fruit leaving no part uncontaminated and making unfit for consumption [17].

Fungi infecting a fresh tomato and secrete some exoenzymes to degrade the cell wall of the tomato and cause invasion in the cells. The productivity of the enzyme for these pathogenic fungal specimens was tested in this study. For active penetration of fungi in vegetables and fruit it must be able to produce enzymes which dissolve the outer plant cell wall which predominantly consists of cellulose and pectin [6]. Filamentous fungi are particularly interesting due to their easy cultivation and high production of extracellular enzymes. These enzymes have commercial application in various industries such as starch, detergents, drinks, food, textiles, animal feed, pulp and paper, leather, chemical and biomedical products [6].

Cellulose is mainly degraded by an enzyme known as cellulase. Cellulase is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds. Although a large number of microorganisms are capable of degrading cellulose, only few of these produce significant quantities of cell free enzymes capable of completely hydrolyzing crystalline cellulose *in vitro*. This is mainly produced by Bacteria, Fungi and Protozoan that catalyse cellulolysis (i.e. hydrolysis) of cellulose [8]. Fungal genera like *Trichoderma* and *Aspergillus* are thought to be cellulose producers [12]. Amylase are the most important enzymes and are of great significance in biotechnology and are commercially important in various starch processing industries. Although amylases are derived from microbial sources generally meet industrial demands. Amylases have been derived from several fungi, yeast, bacteria and actinomycetes but members of the genus *Bacillus* are heterogeneous and they are very versatile in their adaptability to the environment [7, 2].

Industrial production of antibiotics, enzymes, and other substances by growing the microorganisms that produce the product in a submerged culture [14]. In the Solid State Fermentation process the solid substrate not only supplies the nutrients to the culture, but also serves as an anchorage for the microbial cells. The moisture content of the medium changes during fermentation as a result of evaporation and metabolic activities and thus optimum moisture level of the substrate is therefore most important [9].

The aim of this study is to isolate and identify the fungal pathogen in spoiled tomato and determine the amylolytic and cellulolytic activity by submerged and solid state fermentation.

MATERIALS AND METHODS

Collection of Sample

The tomato sample were collected from five different vegetable market in Nagercoil, Kanyakumari District. They were collected in sterile polythene bags separately and transported to the laboratory for further studies.

Isolation and identification of fungal species

The samples were crushed with sterile mortar and pestle and subjected to serial dilution using sterile water. Further the sample were plated Sabourauds Dextrose Agar (SDA) with Streptomycin in supplemented in the media before pouring in the Petri plates to prevent bacterial

growth. The dilution of 10^{-3} , 10^{-4} and 10^{-5} was taken for plating in the media for the tomato samples. Upon incubation for 48 hours, distinct plates inoculated which were identified morphologically by the Lactophenol Cottonblue (LPCB) staining.

Enzymatic study of the fungal samples

The important carbohydrates present in plants is cellulose and starch in the cell as food reserve. For a successful invasion of fungi and cause infection it must be able to dissolve the outer layer by producing extracellular enzymes.

Screening of cellulase activity

Cellulase is the most important building component of plant cell walls. The media prepared consisted of cellulose: 5g/L, Yeast extract: 5g/L, Agar: 15g/L. The media was autoclaved at 121°C for 15 minutes. Then the media was poured in sterile petriplates, one plate for each fungi to be inoculated. Upon solidification, using needle and forceps a bit of the fungal culture was taken and placed onto the media without further disturbing its position. The plates were left for incubation at room temperature for 72 hours.

Screening of amylase activity

Amylase forms an important component of the starch. Amylase is broken down into dextrans and glucose units by the action of amylase enzyme. To demonstrate the amylase activity the media consisted to Himedia Nutrient agar with 2% starch supplemented to it. Further the fungal specimens were inoculated and incubated in the same way.

Production of fungal amylase by submerged fermentation method

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. Allow to cool down to room temperature. Incubate with shaking for 72 hours. After incubation pour the whole content of the flask containing the growing fungus through a funnel fitted with whatmann number 1 filter paper. The filtrate contains the crude amylase and used for further studied.

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) + 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 (1 unit/ml = amount of enzyme which releases 1 μ mole glucose under the assay condition).

Production of fungal cellulose by submerged fermentation method

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. Allow to cool down to room temperature. Incubate with shaking for 72 hours. After incubation pour the whole content of the flask containing the growing fungus through a funnel fitted with whatmann number 1 filter paper. The filtrate contains the crude amylase and used for further studied.

Assay of Cellulase

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) + 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 (1 unit/ml = amount of enzyme which releases 1 μ mole glucose under the assay condition).

Production of fungal amylase by solid state fermentation

For production of amylase, in solid state fermentation, 5g of solid substrate coconut oil cake and 10ml of distilled water was taken in 250 ml Erlenmeyer flask and were inoculated with the culture. Inoculated production media were incubated under static conditions at 28°C and enzyme production was checked after 72 hours incubation. Enzyme was extracted in 50 ml of 0.1m phosphate buffer (pH 6.5) on a rotary shaker at 250 rpm for 30 min. The content was filtered through cheese cloth, filtrate was centrifuged at 8000 rpm for 10 min and clear brown supernatant was used as the enzyme source.

Amylase activity can be found out with the help of glucose standard graph. Enzyme activity was expressed in units (1 unit/ml = amount of enzyme which releases 1 μ mole glucose under the assay condition).

Production of fungal cellulose by solid state fermentation

For production of amylase, in solid state fermentation, 5g of solid substrate saw dust and 10ml of distilled water was taken in 250 ml Erlenmeyer flask and were inoculated with the culture. Inoculated production media were incubated under static conditions at 28°C and enzyme production was checked after 72 hours incubation. Enzyme was extracted in 50 ml of 0.1m phosphate buffer (pH 6.5) on a rotary shaker at 250 rpm for 30 min. The content was filtered through cheese cloth, filtrate was centrifuged at 8000 rpm for 10 min and clear brown supernatant was used as the enzyme source.

Amylase activity can be found out with the help of glucose standard graph. Enzyme activity was expressed in units (1 unit/ml = amount of enzyme which releases 1 μ mole glucose under the assay condition).

RESULT AND DISCUSSION

The present study was undertaken to isolate an efficient enzymes producing microorganisms from the spoiled tomato. Three different isolates were isolated from the spoiled tomato were screened for enzyme activity. Based on the morphological characters and microscopic observation 3 filamentous fungi were observed those are *Aspergillus* species., *Penicillium* species, *Trichoderma* species., *Aspergillus* species the identifying features of this species are coarse black granules against the creamy colony. Globose vesicles with biseriate phialids. Large echimulate jet black conidia in chains [15]. *Penicillium* species, colony morphology is flat granular that are typically blue green, Phialides are formed as blunt tips. Phialides may be arranged in whorls. [10]. *Trichoderma* species unicellular hyaline phialoconidia on short plump flask shaped phialophores. Wide angle of branches of the phialophores and phialides. Clusters of

sub glucose to elliptical conidia in balls at the tip of the phialides [6]. Sulaiman [16] also have isolated three different species of fungi isolated and identified to be associated with fruit rot of tomatoes are *Aspergillus viridae*, *Penicillium digitatum* and *Rhizopus* sp.

Fig.1. Amylase production by fungal species in submerged fermentation

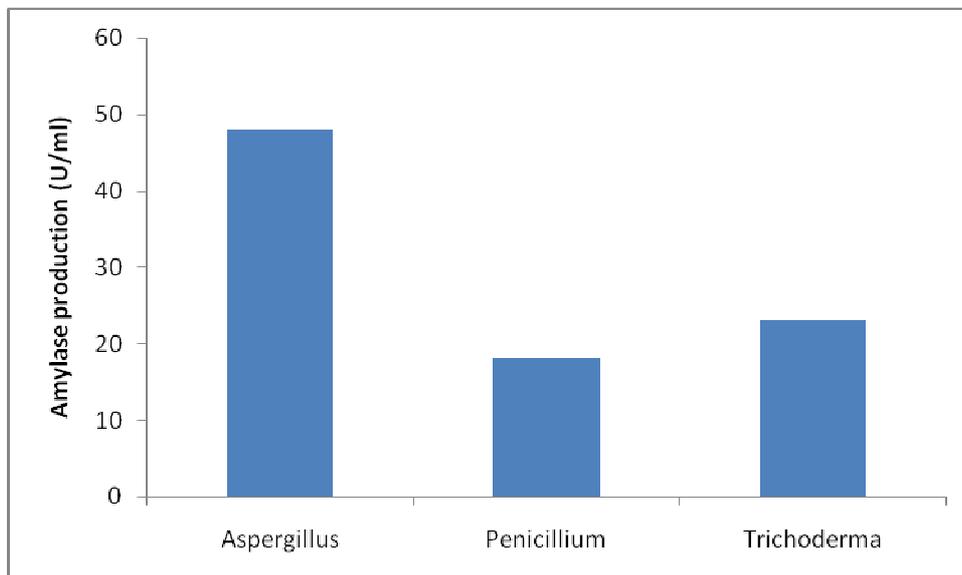
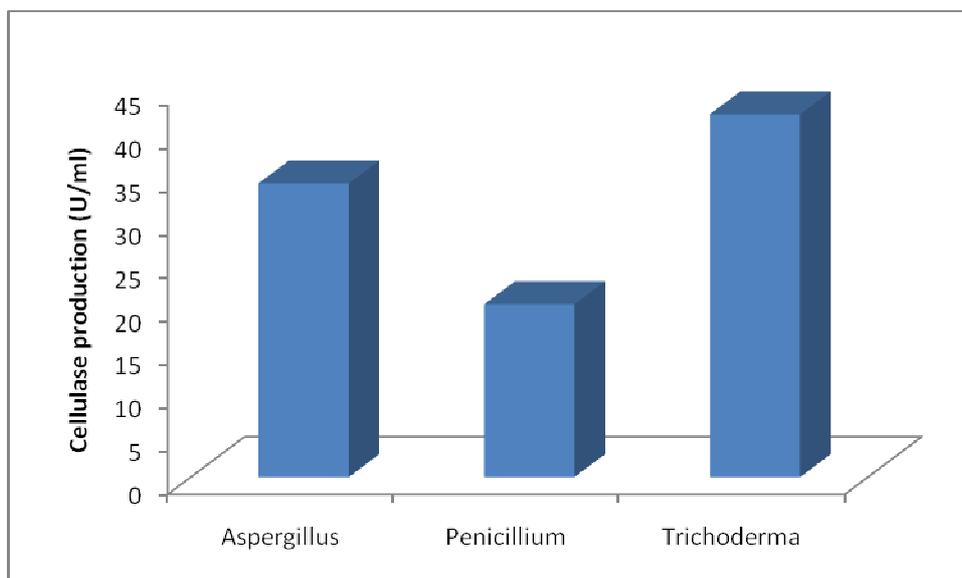


Fig. 2. Cellulase production by fungal species in submerged fermentation



Among the three isolates, *Aspergillus* species more of amylase activity and moderate Cellulase activity was observed. However, for *Trichoderma* species more of cellulose activity, moderate amylase activity were seen. Finally for *Penicillium* species the amylase activity was more followed by Cellulase [13].

In this present study, in submerged fermentation the production of amylase by the fungal species *Aspergillus* was reached maximum of 48/ml at 72hrs of incubation period, *Penicillium* species produced 18U/ml and *Trichoderma* species produced 23U/ml these are shown in Fig.1. The production of Cellulase by the fungal species *Trichoderma* was reached maximum of 42U/ml,

Penicillium species produced 20U/ml and *Trichoderma* species produced 34U/ml at 72 hours of incubation period, these are shown in Fig.2.

Fig.3. Amylase production in solid state fermentation (Coconut oil cake) by fungal species

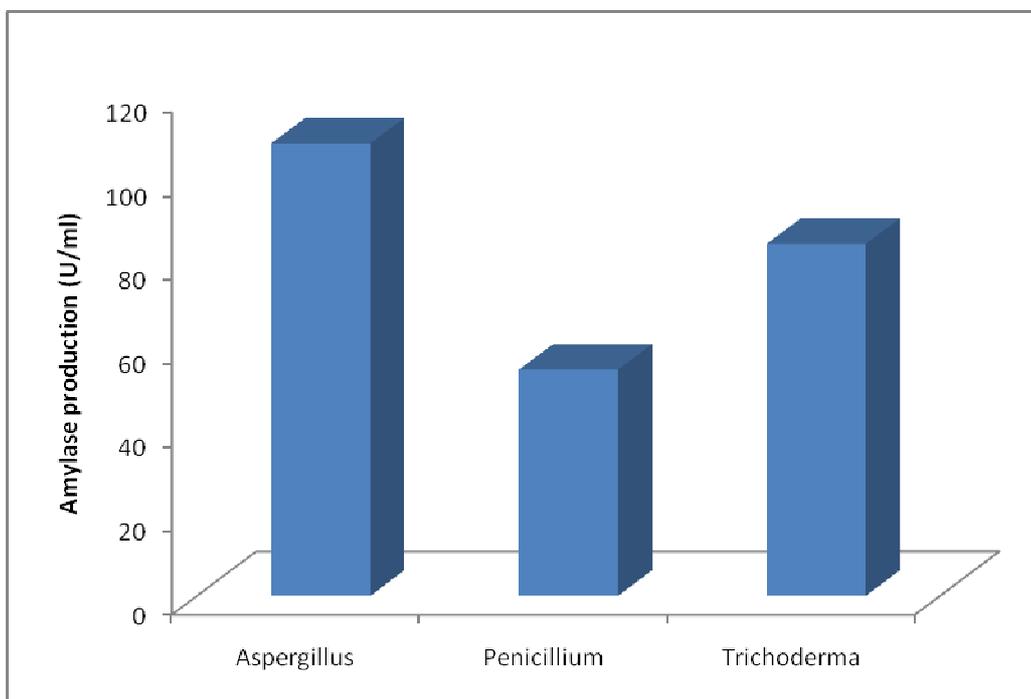
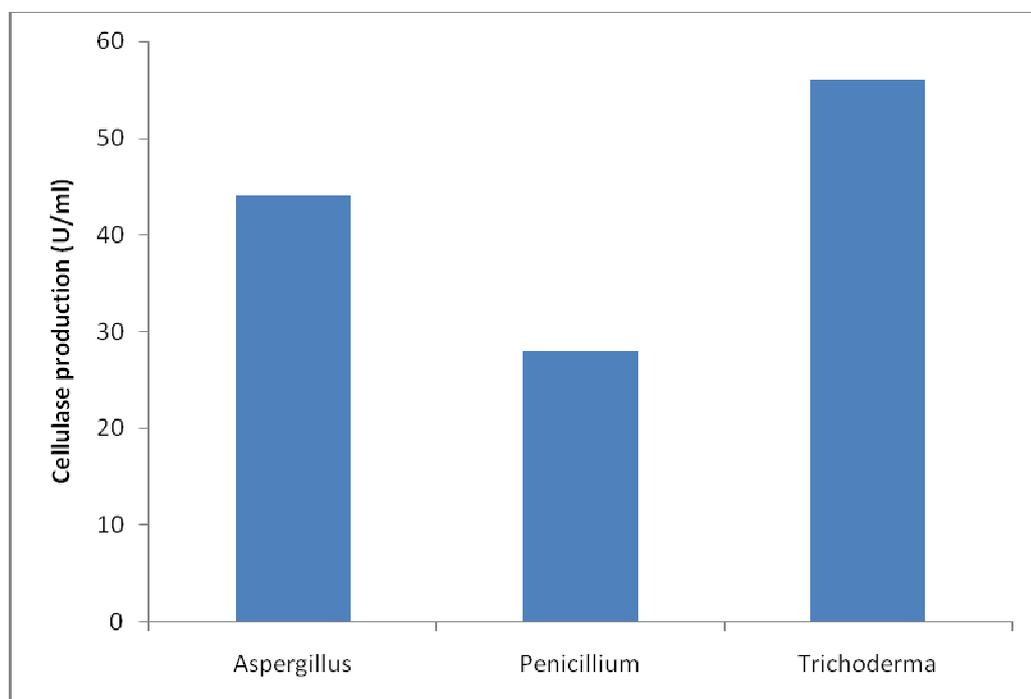


Fig.4. Cellulase production in solid state fermentation (Saw dust) by fungal species



In this present study, In solid state fermentation (Coconut oil cake) the production of amylase by the fungal species *Aspergillus* was reached maximum of 108U/ml, *Penicillium* species 54U/ml and *Trichoderma* species 84U/ml was shown in the Fig.3. The cellulose production by fungal

species in saw dust as a substrate the fungal species *Trichoderma* was reached maximum of 56U/ml, *Penicillium* species 28U/ml and *Aspergillus* species 44U/ml was shown in the Fig.4.

In the present study, among the three fungal species *Trichoderma* species were isolated samples and selected as the major cellulolytic fungal strains for cellulose enzyme production [11]. The results shows clearly that upon successful contact with these fungi a healthy and fresh vegetable or a fruit can easily succumb to infection due to their ability of secreting these exoenzymes. Upon infection these produces potent mycotoxins which can diffuse in the entire food substrate which can result in severe detrimental effect upto the level of being fatal [1].

These fungal organisms are often exploited in the industries because of their ability to produce extra cellular enzymes and thus find its application in various industries such as starch, detergents, drinks, food, textiles, animal feed, pulp and paper, leather, chemical and biomedical products [6].

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