RESEARCH ARTICLE

STUDIES ON ENZYME PROTEASE ACTIVITY PRODUCTION BY COMMON AND DOMINANT MYCOFLORA OF CARROT (*DAUCUS CAROTA L.*)

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Abstract:

The vegetables are the most important group of plants farming important and dispensable part of our daily diet. Carrots are rich source of carotene and vitamins-A. Most of the fungi that are initially observed on whole vegetable surface are soil inhabitant. They are important source of dietary protein essential amino acids and micro nutrients such as calcium and iron. About 88% of protein consume in India are of vegetable origin independence on animal protein being very less. Biodeterioration of vegetable has been attributed main lead to protease activity in additional to other hydrolytic enzyme secret by mould associated with the vegetable, therefore extensive study undertaken on the production of protein by the vegetable fungi. Determination of protease activity was done with the help of cup plate method adopted by Hislop and Rajamoni. Protease production by *Alternaria tenuis* Auct., *Aspergillus flavus* Link ex. Fr., *Aspergillus niger* van Tiegh., *Curvularia lunata* (Wakker) Boedijn., *Drechslera tetramera* Subram. & Jain. and *Fusarium moniliforme* Sheldon. was studied by growing them on liquid Carrots powder nitrate medium (CN medium) separately. This study present in paper economical approach for the bio conservation of vegetable Carrot protease activity of enzyme. The proteases essay by complete method and result are presented table. The common and dominant seed born of fungi produce protease enzyme in variable quantity which help the fungi degrade the seeds and ultimately affect seed quality yield. Indian police variety of biochemical physiological and regulatory functions protease have a place key role. Spoilage generally leads to undesirable change in colour, order flavour, texture they may occurs during cultivation transportation or storage.

Keywords: Isolation, Carrot, Mycoflora, Protease Activity, Post Harvest Fungi.

Introduction:

Carrots are eaten both fresh and cooked. Carrots have long been associating with several benefits the vitamin A in carrots is beneficial for eye health. Orange carrots are a good source of substance called carotene. Carrots are also a great source of dietary fibres which helps to reduce blood sugar and cholesterol. Carrots are 100 species have been reported to infect. Out of 30% species are commonly associated with Carrot spoilage because an environmental condition, Carrot variety, handling and storage. Carrots total Fourteen fungi found on roots and seeds. Most of the fungi cause Decay and rots (Kunte and Yawalkar, 1991). The biodeterioration of vegetables and their seeds show many changes in their contents (Verma *et al.,* 1991). Vegetable waste as input for proteins production using *Aspergillus niger* wasted like Cabbage, brinjal, Pumpkin, etc maximum enzyme activity (Chamraj *et al.,* 2011). Bio-deterioration of Carrots and their seeds directly related with the toxin and enzyme production by the associated fungi. The objective of present investigation study in paper economical approach for the bio-conservation of vegetable waste for production activity of enzyme protease activity of common and dominant vegetable fungi studied. For this the vegetable fungi were grown on liquid Carrot root powder nitrate (CN) medium over a period of ten days. After incubation period, culture filtrate (crude enzyme preparation) was collected. The protease activity was assayed in agar solidified basal Glucose gelatine agar (GGA) medium by cup plate method. The results are presented in table 1.

Materials and Methods:

1. Preparation of spore suspension:

Spore suspension of common and dominant vegetable mycoflora was prepared separately by adding 10 ml of sterile distilled water into the sporulating pure cultures vegetable mycoflora namely *Alternaria tenuis* Auct., *Aspergillus flavus* Link ex. Fr., *Aspergillus niger* van Tiegh., *Curvularia lunata* (Wakker) Boedijn., *Drechslera tetramera* Subram. & Jain. and *Fusarium moniliforme* Sheldon. maintained on PDA slants for seven days at room temperature. The slants were shaken and content was filtered through muslin cloth to separate mycelium and spore. The filtrate thus obtained was used as spore suspension.

2. Composition of media reagents and indicators used during present study

Carrot root powder nitrate (CN) medium

Carrot root powder-10 gm.

KNO_3-2.5 gm

KH^2PO_4- 1gm

$MgSO_4.7H_2O$-0.5 gm.

Distilled water1000 ml

3. Basal medium for protease assay

Glucose-10 gm

Agar-2 gm

Gelatin-1 gm

Distilled water-100 ml

4. Protease production:

Protease production by some common and dominant vegetable mycoflora such *as Alternaria tenuis* Auct., *Aspergillus flavus* Link ex. Fr., *Aspergillus niger* van Tiegh., *Curvularia lunata* (Wakker) Boedijn., *Drechslera tetramera* Subram. & Jain. and *Fusarium moniliforme* Sheldon. was studied by growing them on liquid Carrots powder nitrate (CN) medium 25 ml of the CN medium was poured in 100 ml conical flasks separately and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with spore suspension of test vegetable fungi prepared from seven day old culture grown on PDA slants. These flasks were incubated for 10 days at room temperature. After incubation the contents were filtered through Whatman filter paper No.1 to remove fungal mat and the liquid part was collected in presterilized bottles and used as crude enzyme preparations for the protease assay by cup plate method and the results are presented in Table

5. Protease assay by cup plate method:

Determination of protease activity was done with the help of cup plate method, adopted by Hislop *et al.* (1982) and Rajamani *et al.* (1988). A basal medium was prepared containing 2% (w/v) agar and 1% (w/v) gelatin. pH of the medium was adjusted at 5.6. The medium was sterilized at 15 lbs pressure for 20 minutes. 15 ml of medium was poured in presterilized Petriplates under aseptic conditions. 6 mm diameter cavities (cups) were made in the center of the solidified agar plate with No. 4 cork borer. About 0.5 ml of culture filtrate (crude enzyme preparation) was poured in the cavity. The plates were incubated at room temperature for 24 hrs. 15% Hgcl2 in 7M HCl was added to the plates. After 10 minutes a transparent zone indicating hydrolysis of gelatin by extra cellular proteolytic enzymes was observed. The diameter of the transparent zone was used as a measure (mm) of protease activity and non-appearance of clear zone considered absence of protease in the culture filtrate (crude enzyme preparation).

Table 1: Protease activity of common and dominant vegetable fungi grown on liquid Carrot (*Daucus carota* L.) root powder nitrate (CN) medium (After ten days of incubation, by cup plate method).

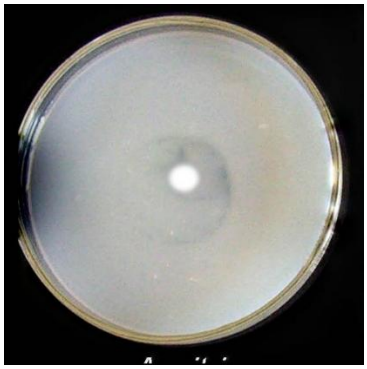
Sr. No.	Common and dominant vegetable fungi	Protease activity zone (mm)
		Glucose-gelatin agar (GGA) Medium
1.	*Alternaria tenuis* Auct.	15
2.	*Aspergillus flavus* Link ex, Fr.	14
3.	*Aspergillus niger* van Tiegh	15
4.	*Curvularia lunata* (Wakker) Boedijn	13
5.	*Drechslera tetramera* Subram. & Jain	23
6.	*Fusarium moniliforme* Sheldon	22

Results and Discussion:

The present investigation confirms that all common and dominant fungal species associated with carrot (*Daucus carota* L.) possess extracellular protease activity, although the degree of enzyme production varies considerably. The largest proteolytic zone was produced by *Drechslera tetramera* (23 mm), indicating strong secretion of hydrolytic enzymes. Fungi

of the genera *Drechslera* and *Fusarium* are widely reported as aggressive colonizers of plant tissues due to their ability to break down complex organic substrates using proteases and other cell-wall degrading enzymes (Sutton *et al.,* 2019). Comparable high activity in *Fusarium moniliforme* (22 mm) suggests efficient nutrient acquisition and rapid degradation ability in carrot medium

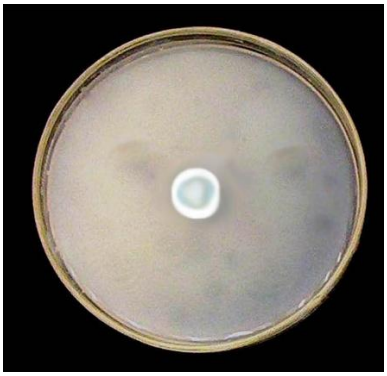
Alternaria tenuis



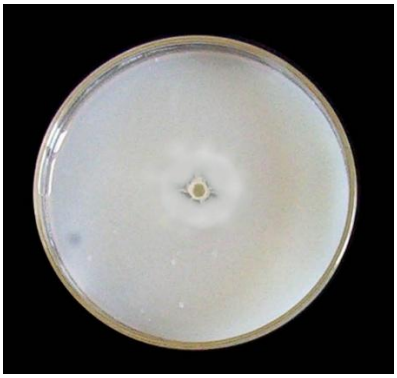
Aspergillus flavus

Aspergillus niger

Curvularia lunata



Drechslera tetramera



Fusarium moniliforme

Plate 1: Protease activity by common and dominant fungi of vegetables

Moderate protease activity observed in *Alternaria tenuis* and *Aspergillus niger* (15 mm) is consistent with earlier findings that *Alternaria* and *Aspergillus* spp. are reliable producers of extracellular enzymes under carbohydrate-rich and slightly acidic conditions (Bello *et al.,* 2018). *Aspergillus* species are frequently used in industrial enzyme production, and nutrient supplementation,

especially nitrogen sources, has been shown to increase microbial protease yield (Kumar & Tak, 2021).

The lowest activity exhibited by *Curvularia lunata* (13 mm) may reflect slower growth kinetics or reduced ability to utilize carrot-based substrates. Variation in protease production among fungal isolates is commonly attributed to differences in genetic regulation, substrate specificity, and environmental factors such as pH and aeration (Mehta & Satyanarayana, 2016). The results demonstrate that carrot tissue provides an effective medium for screening protease-producing fungi, aligning with reports that vegetable waste substrates can serve as low-cost raw material for microbial enzyme production (Singh *et al.,* 2020).

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