A COMPARATIVE STUDY OF LYTIC ENZYMES ACTIVITY EXTRACTED FROM DIFFERENT *TRICHODERMA* SPECIES AND THEIR ROLE IN INDUSTRIES

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Abstract: We have selected two strains (T. viride and TB1) of Trichoderma species to compare its enzymatic activity. T. viride collected from NCIM, Pune and TB1 (isolated from banana soil rhizosphere). Trichoderma strains were cultivated by using novel neem cake wheat bran substrate for SSF. The strains were then harvested and the enzymes were extracted using different solvents. Enzyme activity was analyzed by plate well diffusion assay technique and the observations suggested TB1 enzymes shows higher enzymatic activity than other strain (T. viride).

KEYWORDS: Trichoderma, Enzymes, Industries.

INTRODUCTION: *Trichoderma* species produces a wide array of enzymes. Some strains are used industries to produce a large quantity of enzymes.*Trichoderma*produces various types of enzymes like chitinase, cellulases, hemicellulase, protease, glucanases, xylanase, amylase, lipase etc. Our recent study aimed to study on lytic enzymes produced by isolated *Trichoderma*and its significance role in agriculture and other industries. Mainly focused on **chitinases, lipases, amylases** and **proteases** enzyme. The extracellular hydrolytic enzymes played important role in mycoparasitism and antibiosis. *Trichoderma* species T. harzianum, T. viride, T. ressei are known as producers of extracellular hydrolytic enzymes. Enzymes play an important role in soil management, organic matter decomposition and nutrient cycling. Some enzymes only facilitate the breakdown of organic matter (e.g., hydrolase, glucosidase), while others are involved in nutrient mineralization (e.g., amylase, urease, phosphatase, sulfates).

MATERIALS AND METHODOLOGY:

Micro Organism- The strain used for lytic enzymes production was T. viride and TB1. T. viride collected from National Collection of Industrial Microbiology (NCIM) Pune, provided by R&D, Kilpest India Limitedand TB1 isolated from banana soil rhizosphere.

Collection of Soil sample: Soil samples were collected from the field of Kilpest India Limited, Bhopal, India. Rhizosphere soil was taken from different plants (Banana, Garlic, Chick pea and Radish plant) from the 5-12 cm depth.

Isolation of Trichoderma strain (TB1) from Soil Rhizosphere: Potato Dextrose Agar media was prepared and autoclaved at 121º C, 15 psi for 20 minutes. Hot media was poured into pre- sterilized petri plates using aseptic condition the plates were allowed to cool causing media to solidify in plates. 1gm of soil was dissolved in 10 ml of water for making serial dilution. Serial dilution series was prepared. Aseptically 20µl of soil suspension were transferred into PDA plates and spreaded uniformly. Plates were incubated at 28ºC for 5-6 days. After the 5-6 days number of colonies were observed in PDA plates. Morphological study was done. Colonies that produced green colour conidia was picked. Subsequently sub culturing was done to obtain pure cultures.

Solid state fermentation (SSF) of T. viride and TB1 using wheat bran and neem cake: Wheat bran and neem cake used as a substrate for SSF. Wet substrate mixture was transferred in 152 X 152 mm plate and autoclaved at 121 ºC at 15 psi for 20 minutes. After sterilization, the substrate were aseptically inoculated with 5 days old culture plate of T. viride and TB1 and plates were incubated at 28 ºC in BOD for 7 days.

Enzyme Extraction from Trichoderma strains (T. viride and TB1) in different solvents: T.viride and TB1 was harvested from culture plate and grinded using electrical heavy duty grinder. Approx. 30 gm of Trichoderma strains was mixed with 60 ml of different solvents to prepare a thick paste which transferred to four 250 ml flasks kept on shaker for 2 hour at 100 rpm. Then

slurry was filtered by using muslin cloth and filter paper. This filtrate was transferred into eppendroff tube and then centrifuged for 1 min at 13000 rpm. Supernatant was collected and used for the enzyme assay.

Determination of enzyme activity of crude enzyme fractions of T. viride and TB1 by using Plate well diffusion assay

Protease Enzyme assay: Plates of Gelatin Agar media were prepared under the aseptic condition. Formed wells in each plates using sterilized cork borer. Inoculated different volumes (40 µl and 50 µl) in wells and incubated at room temperature for 24 and 48 hours to observe enzyme activity. **Indicator-** Mercuric chloride was used as indicator. Indicator was poured in media plate, uniformly distributed. After the 20 min zones were observed. The resulting diameters of the clearing zone were measured by using ZOI (zones of inhibition) scale.

Amylase Enzyme assay: Plates of Starch Agar media were prepared under the aseptic condition. Formed wells in each plates using sterilized cork borer. Inoculated different volumes (40 µl and 50 µl) in wells and incubated at room temperature for 24 and 48 hours to observe zonal activity. **Indicator-** Iodine solution was used as an indicator. Indicator was poured in media plate, uniformly distributed. After the 15 min zones was observed. The resulting diameters of the clearing zone were measured byusing ZOI (zones of inhibition) scale.

Cellulase Enzyme assay: Plates of CMC Agar media were prepared under the aseptic condition. Formed wells in each plates using sterilized cork borer. Inoculated different volumes (40 µl and 50 µl) in wells and incubated at room temperature for 24 and 48 hours to observe zonal activity. **Indicator-** Congo red solution used as indicator and 1M Sodium chloride used as a counter stain for Cellulase enzyme activity. Congo red solution was poured in media plate, uniformly distributed for 10 min. Then counter stain was poured in plate for 10 min. Zones was observed. The resulting diameters of the clearing zone were measured by using ZOI (zones of inhibition) scale.

Chitinase Enzyme assay: Plates of Chitinase detection media were prepared under the aseptic condition. Formed wells in each plates using sterilized cork borer. Inoculated different volumes (40 µl and 50 µl) in wells and incubated at room temperature for 24 and 48 hours to observe zonal activity. **Indicator-** Bromocresol purple solution was used as an indicator. Indicator was poured in media plate, uniformly distributed. After the 15 min zones was observed. The resulting diameters of the clearing zone were measured by using ZOI (zones of inhibition) scale.

Lipase Enzyme assay: Plates of Lipase detection media were prepared under the aseptic condition. Formed wells in each plates using sterilized cork borer. Inoculated different volumes (40 µl and 50 µl) in wells and incubated at room temperature for 24 and 48 hours to observe zonal activity.**Indicator-** Methyl red solution was used as an indicator. Indicator was poured in media plate, uniformly distributed. After the 15 min zones was observed.The resulting diameters of the clearing zone were measured byusing ZOI (zones of inhibition) scale.

RESULTS AND DISCUSSION: The study aimed at choosing a better strain of Trichoderma species, by checking the activity of lytic enzymes. We collected soil samples from different plants soil rhizosphere (Banana, garlic, radish, chickpea) from field of Kilpest India Limited, Bhopal. We successfully isolated Trichoderma strain TB1 from banana soil rhizosphere and other strains Trichoderma viride revived on PDA plates, were provided by R&D, Kilpest India Limited Bhopal.

SSF was done for extracting the enzymes using wheat bran and neem cake. Enzyme was successfully extracted in different solvents (phosphate buffer, methanol, 1N Saline and 10% ethanol) using filtration and centrifugation process. The activity of lytic enzymes was successfully observed in plate well diffusion assay. We prepared different agar media for different enzymes for example gelatin agar media used for protease enzyme assay and starch agar media used for amylase assay. We compared the activity of lytic enzymes of Trichoderma strains TB1 and T. viride by determining zones diameter. The newly isolated Trichoderma strain TB1 showed better enzymatic activity in phosphate buffer than T. viride. TB1 showed higher activity of cellulase, amylase and protease enzymes which is useful in various industries.

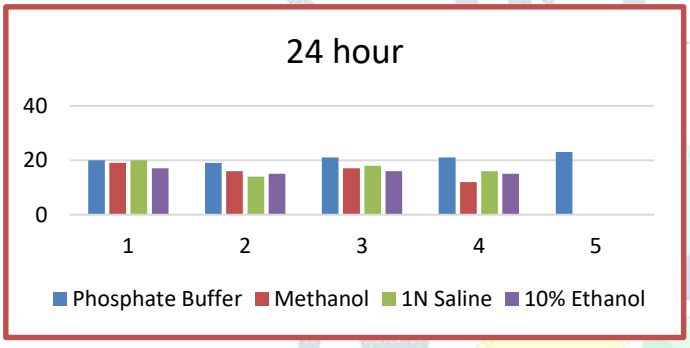


(A) (B) (C)

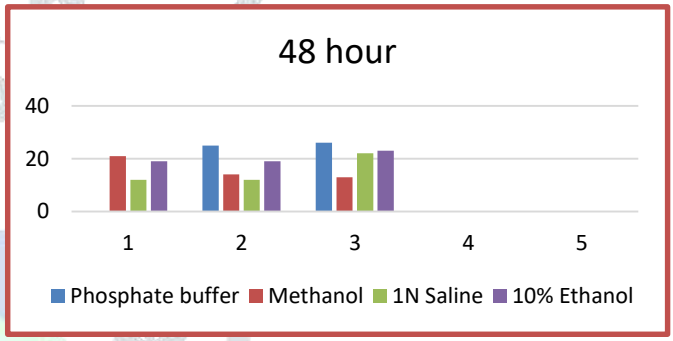
Fig. (A) representing amylase enzyme activity, (B) representing cellulase enzyme activity, (C) representing protease enzyme activity.

Table 1- Represented T. viride enzymes activity in different solvents at different incubation time.

Solvent	Incubation time	Strain	Enzymes				
			Protease	Amylase	Cellulase	Lipase	Chitinase
Phosphate Buffer	24 hour	T. viride	20 mm	19 mm	21 mm	21 mm	23 mm
Methanol			19 mm	16 mm	17 mm	12 mm	-
1N Saline			20 mm	14 mm	18 mm	16 mm	-
10% Ethanol			17 mm	15 mm	16 mm	15 mm	-
Phosphate Buffer	48 hour	T. viride	-	25 mm	26 mm	-	-
Methanol			21 mm	14 mm	13 mm	-	-
1N Saline			12 mm	12 mm	22 mm	-	-
10% Ethanol			19 mm	19 mm	23 mm	-	-



(A)

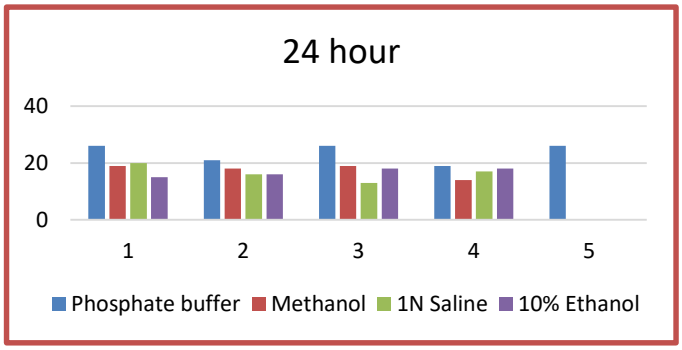


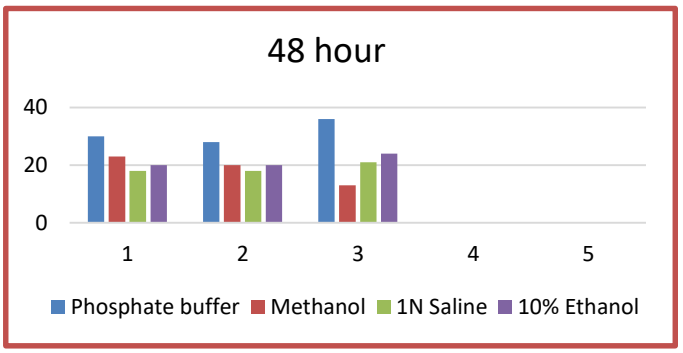
(B)

Graph A and B represented 24 hour and 48 hour enzymatic activity of T. viride in different solvents. 1, 2, 3, 4, 5 numbers representing Protease, amylase, cellulase, lipase and chitinase enzyme activity respectively.

Table 2- Represented TB1 enzymes activity in different solvents at different incubation time.

Solvent	Incubation time	Strain	Enzymes				
			Protease	Amylase	Cellulase	Lipase	Chitinase
Phosphate Buffer	24 hour	TB1	26 mm	21 mm	26 mm	19 mm	26 mm
Methanol			19 mm	18 mm	19 mm	14 mm	-
1N Saline			20 mm	16 mm	13 mm	17 mm	-
10% Ethanol			15 mm	16 mm	18 mm	18 mm	-
Phosphate Buffer	48 hour	TB1	30 mm	28 mm	36 mm	-	-
Methanol			23 mm	20 mm	13 mm	-	-
1N Saline			18 mm	18 mm	21 mm	-	-
10% Ethanol			20 mm	20 mm	24 mm	-	-





(C) **(D)**

Graph C and D represented 24 hour and 48 hour enzymatic activity of TB1 in different solvents.
1, 2, 3, 4, 5 numbers representing Protease, amylase, cellulase, lipase and chitinase enzyme activity respectively.

CONCLUSION: The newly isolated Trichoderma strains TB1 showed higher enzymatic activity in phosphate buffer than T. viride in laboratory. Enzymes produced by Trichoderma useful in plant growth (protection from pathogens, antagonistic activity and to increase soil fertility), Textile Industry, Paper Industry and Food Industry.Cellulase enzyme useful in Paper and pulp industry, agricultural industry, Food processing industry, animal feed industry, detergent industry and textile industry (Ramesh et al 2011). Protease enzyme useful in detergent, leather, dairy, baking and pharmaceutical industries (Rao et al 1998). Amylase enzyme useful in Food, fermentation, textile and paper industries (Lin et al 1997, Pandey et al 2000). Lipase enzyme useful in detergent, dairy, agro chemical, paper, nutrition, cosmetic and pharmaceutical processing and industry (Sharma et al 2001). The application of enzymes isolated from Trichoderma strains may become a reality in future as they can be produced cheaply in large quantities on an industrial scale.

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