CHAPTER 3

Evaluation of plastic degrading fungal enzymes

Enzymes responsible for degradation of polyethylene were identified by literature survey as Sowmya et al. (2015); Temporiti et al. (2022); Skariyachan et al. (2015) and Bhardwaj et al. (2013). In order to confirm the presence of responsible enzymes, qualitative tests were conducted to assess the activity of laccase, manganese peroxidase (MnP), lipase, esterase, and protease in *A. oryzae* (SA15) and *F. solani* MN201580.1 (SA17). These enzymes are known to play a role in the degradation of the carbon backbone of polyethylene. The tests aimed to determine whether SA15 and SA17 produce these enzymes, indicating their potential involvement in the degradation process. Understanding the enzyme profile of these fungi is crucial for elucidating the mechanisms underlying their degradation capabilities and guiding future optimization efforts.

The evaluation of enzymes involved a two-step process, starting with qualitative analysis followed by the quantification of enzymes. Initially, the presence of responsible enzymes was assessed in both fungal strains using plate assays specific to each enzyme. Following the qualitative analysis, the enzymes were quantified to determine their activity in the selected fungal strains. This involved the extraction of enzymes from the fungal strains and subsequent assays specific to each enzyme. The quantification of enzyme activity provided valuable information about the enzymatic potential of the fungal strains in degrading polyethylene.

By the end of this experiment, the total amount of enzyme activity of the fungal strains was quantified. This quantitative data is essential for understanding the degradation process carried out by the selected fungal strain, as it provides insights into the enzymatic activity and the potential of the strain to degrade polyethylene.

METHODOLOGY

Screening of fungal isolates for proteolytic, lipolytic, esterolytic and ligninolytic enzyme activities

Enzymes responsible for polyethylene were qualitatively checked for their presence in *A. oryzae* (SA15) & *F. solani* MN201580.1 (SA17) fungal strains by performing respective plate assay methods. All enzymatic activities were screened with three replicates.

-Protease enzyme

Both strains SA15 & SA17 were grown on solid media containing 0.5% skim milk powder as source of casein, incubated at 28±1° C and after 48 hours development of the proteolysis zone on the casein agar plate was considered as positive enzymatic activity (Ponnuswamy and Samuel, 2013).

-Lipase enzyme

Lipase activity was screened on Rhodamine olive oil agar medium. Olive oil (31.25 ml) and Rhodamine B (0.001% [wt/vol]) solution were added to Czapek agar medium and stirred vigorously for 1 min (Ramnath et al., 2017). Strains were inoculated on this media & incubated at 28±1° C. Production of lipase enzyme was detected by irradiating plates with UV light at 350 nm, a fluorescent halo indicated lipolytic activity (Valeria et al., 2003).

-Esterase enzyme

Esterase activity was determined on a basal medium containing 0.5% (wt/vol) peptone, 0.3% (wt/vol) yeast extract and 2% agar supplemented with 1%, 2% and 3% tributyrin. Selected strains were inoculated by boring 5mm wells and incubated at 28±1° C for 7 days (Ramnath et al., 2017). Culture replicates were exposed to Lugol's iodine solution to enhance the clear halos around the colonies (Faiz et al., 2007; Sayali et al., 2013).

-Laccase & Manganese peroxidase enzyme

Enzyme activity was assayed by substituting malt extract agar medium with 3% tannic acid, cultures were inoculated and kept for incubation at 28±1° C for 7 days. The activity was assessed by observing development of dark brown color around the fungal colonies (Bains, 2006).

Evaluation of proteolytic, lipolytic, esterolytic and ligninolytic enzyme activities

A. Preparation of Fungal Enzymes: Different growth media were prepared for respective enzymatic activities. The medium was inoculated with 9mm disc of 10 days old culture under aseptic condition and incubated at desired temperature for the desired incubation period.

To check the effect of fungal inoculum size on the enzyme production, the experimental set ups were inoculated with single disc and three discs. After respective incubation period fungal mycelium in the flask were homogenized by laboratory hand blender and filtered through pre-weighed Whatman paper no. 1 to collect the culture filtrate and fungal biomass. All enzymatic activities were performed with three replicates for each incubation period and results reported as the mean of replicates.

-Protease enzyme

For the evaluation of proteolytic enzyme activity Casein (10 gm/l) broth media with peptone (5 gm/l) was prepared and autoclaved at 121˚C temperature and 15psi pressure for 20 minutes. The collected culture filtrate was centrifuged at 5000 rpm for 20 minutes and supernatant used as crude enzyme extract for the quantification (Mais E Ahmed, 2018).

-Lipase & Esterase enzyme

Lipolytic enzymes were evaluated by using mineral salt medium containing 0.5% yeast extract and 1% olive oil. The mineral salt solution contained: KNO_3 (2 gm/l), $MgSO_4.7H_2O$ (0.5 gm/l), K_2HPO_4 (1 gm/l), $ZnSO_4.7H_2O$ (0.44 gm/l), $FeSO.7/H_2O$ (1.1gm/l) and $MnSO_4.7H_2O$ (0.2 gm/l) (Valeria et

al., 2003). At time intervals, the enzyme was extracted from the growth medium by centrifugation at 10,000 rpm for 15 minutes and supernatant was used as the crude enzyme.

-Laccase & Manganese peroxidase enzyme

Ligninolytic enzymes were prepared in Malt extract broth medium containing 30 gm Malt extract powder in 1000 ml Distilled water. The removed culture filtrates at desired time intervals were considered as crude enzyme for the quantification (Bettin et al., 2009).

B. Enzyme assays:

Enzyme activities were quantified by preparing three replicates of each reaction mixtures. Blank control mixtures were prepared simultaneously without adding enzyme extract.

-Protease enzyme

Protease enzyme was assayed with the reaction mixture containing 0.5% casein in 1.47 ml of 0.1 M Tris-Hcl buffer, 8.5 pH and 0.05 ml crude enzyme was incubated at 50°C for 10 minutes. 1.5 ml of cold 10% Trichloro Acetic Acid (TCA) to stop the reaction and kept for one hour at room temperature. The reaction mixture was centrifuged at 8000 rpm for 5 minutes and absorbance of the supernatant was read at 280 nm (Kumar and kalpana, 2008).

-Lipase enzyme

Lipase enzyme activity was quantified using *p*-nitrophenyl palmitate (*p*-NPP) as a substrate according to Nawani et al. (2006) methodology with some modifications. The mixture contained 0.3 ml 0.05 M Phosphate buffer pH 8, 0.1 ml 0.8 mM *p*-NPP and 0.1 ml enzyme extract was incubated at 37° C temperature. After 10 minutes of incubation 1 ml ethanol was added to terminate the reaction. The final yellow colored product was read spectrophotometrically at 410 nm.

-Esterase enzyme

Enzyme activity was measure with *p*-nitrophenyl valerate (*p*-NPV) as a substrate prepared in 50 mM Tris-HCl (pH 7.2) containing 2% acetonitrile. The reaction mixture was prepared taking 10µl of 1 mM *p*-NPV, 25 µl of 50 mM Tris-HCl, 25 µl crude enzyme and 440 µl of distilled water; later the final solution was spectrophotometrically measured at 400 nm (Manco et al., 1994).

-Laccase enzyme

Activity was determined by using 500 µM 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) as a substrate prepared in 50 mM Sodium acetate buffer at pH 4.5. 20 µl of enzyme extract was added to 580 µl ABTS solution and absorbance was recorded at 418 nm (Qi-HE *et al.,* 2011).

-Managanese peroxidase enzyme

MnP enzyme was quantified according to the procedure given by Castillo et al. (1994), 0.167 mM 3-methyl-2-benzothiazolinone hydrazone (MBTH) and 2.37 mM 3-(dimethylamino) benzoic acid (DMAB) as substrates prepared in 0.1 M succinic-lactic acid buffer at pH 4.5. Total one milliliter of

reaction mixture contained 417.5 µl MBTH, 417.5 µl DMAB, 100 µl 3 mM $MnSO_4$, 50 µl enzyme solution and 15 µl 4 mM H_2O_2. The solution producing a purple-colored reaction was measured at 590 nm (Hossain and Anantharaman, 2006).

RESULT & DISCUSSION

Screening of fungal isolates for proteolytic, lipolytic, esterolytic and ligninolytic enzyme activities

Previous reports portray that protease, esterase, lipase, and also ligninolytic enzymes such as laccase and manganese peroxidase (MnP) are significantly associated with polyethylene degradation. However, there are not many research studies dedicated to the responsible enzymes in the process of breaking down this complex structure.

A study conducted by Yao et al. (2022) provided experimental evidence of laccase in degradation of PE polymers. The enzyme laccase plays a vital role in oxidation of the polyethylene hydrocarbon backbone. The reduction in molecular weight of polyethylene indicates biodegradation of polyethylene (Sivan, 2011). As reported by Santo et al. (2013), incubation with laccase enzyme can lead to the reduced molecular weight of polyethylene and increased keto carbonyl index, indicating the breakdown of large polyethylene molecules by scission and oxidation reactions.

Fungal laccases and peroxidases, generally degrade lignin component, and the enzymes have shown significant results in degrading PE material. Fungal hydrolases such as lipase, esterase & protease are capable of modifying the plastic surface and increasing its hydrophilicity (Temporiti et al., 2022). Lipases are known to degrade fat, oil and grease (FOG) waste from municipal wastewater (Masse et al., 2001). According to Gricajeva et al. (2022), esterases hydrolyze short-chain of carbon ($C < 10$) which are usually water-soluble acyl esters, whereas lipases are known to hydrolyze long-chain of carbon ($C > 10$) water-insoluble acyl esters.

Proteases are known to breakdown different types of polymers like polylactic acid (Ozsagiroglu et al. 2012) and polyethylene terephthalate (PET) (Hu et al., 2021). Fungal strains with genus *Aspergillus*, *Trichoderma*, *Penicillium*, *Fusarium* (Loredo-Treviño et al. 2011; Cosgrove et al. 2007), *Phaenarochete* (Shimao 2001), *Pestalotiopsis* (Russell 2011) *Rhizopus*, *Mucor* (Souza et al. 2015) are some of the imperative fungal species for secreting proteases to breakdown polymeric chain.

The presence of enzymes in selected fungal strains *A. oryzae (*SA15) and *F. solani* MN201580.1 (SA17) were qualitatively checked by inoculating fungal isolates on various agar mediums. Culture plates were observed for one week to observe the fungal growth and the clear zone below fungal colonies. Observations revealed that *SA17 & SA15* showed all five-enzyme activities within one week of incubation period. Malt extract agar medium consisting of 3% tannic acid started developing brown color around the colonies from second day of inoculation indicating the ligninolytic activity. *SA17*

inoculated plates showed prominent dark-colored pigmentation around the colonies (Figure.26-C) compared to *A. oryzae* (Figure.26-F). Sowmya et al. (2015) reported presence of laccase and Mnp enzyme activity in an unknown species *Fusarium*.

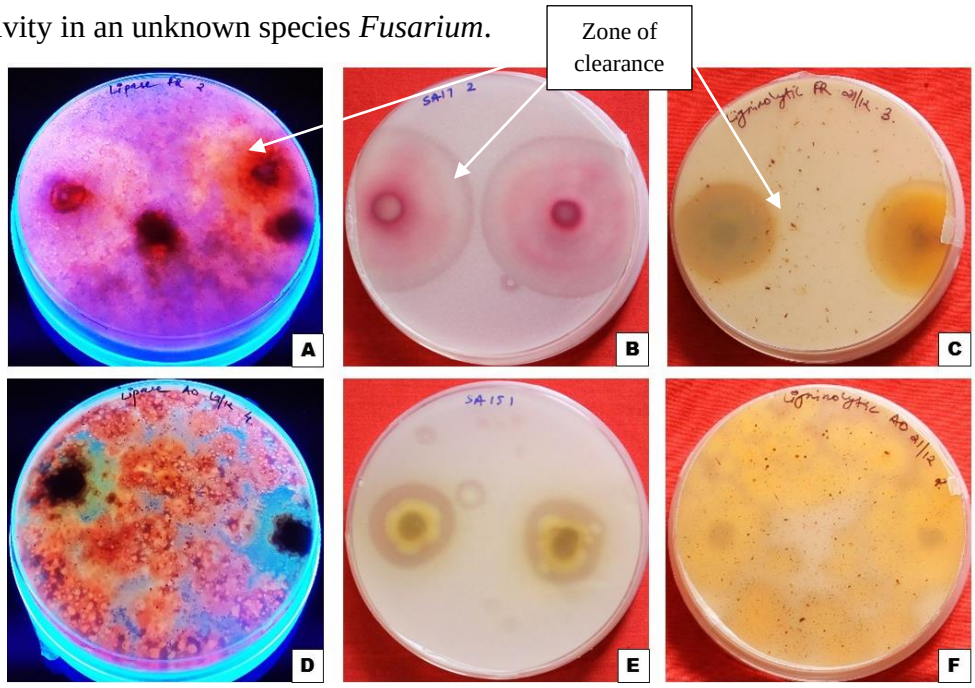

Figure.26. Qualitative analysis of Lipolytic, Proteolytic & Ligninolytic enzyme activity by fungal strains *F. solani* & *A. oryzae*

A&D: Fluorescent orange colored ring around the colonies of *F. solani* & *A. oryzae* respectively- Lipase plate assay (arrow), B&E: Casein hydrolysis zone around the colonies of *F. solani* & *A. oryzae* respectively (arrow)- Protease plate assay, C&F: Dark brown coloration below the fungal growth of strains *F. solani* & *A. oryzae* respectively- Ligninolytic enzyme plate assay (arrow)

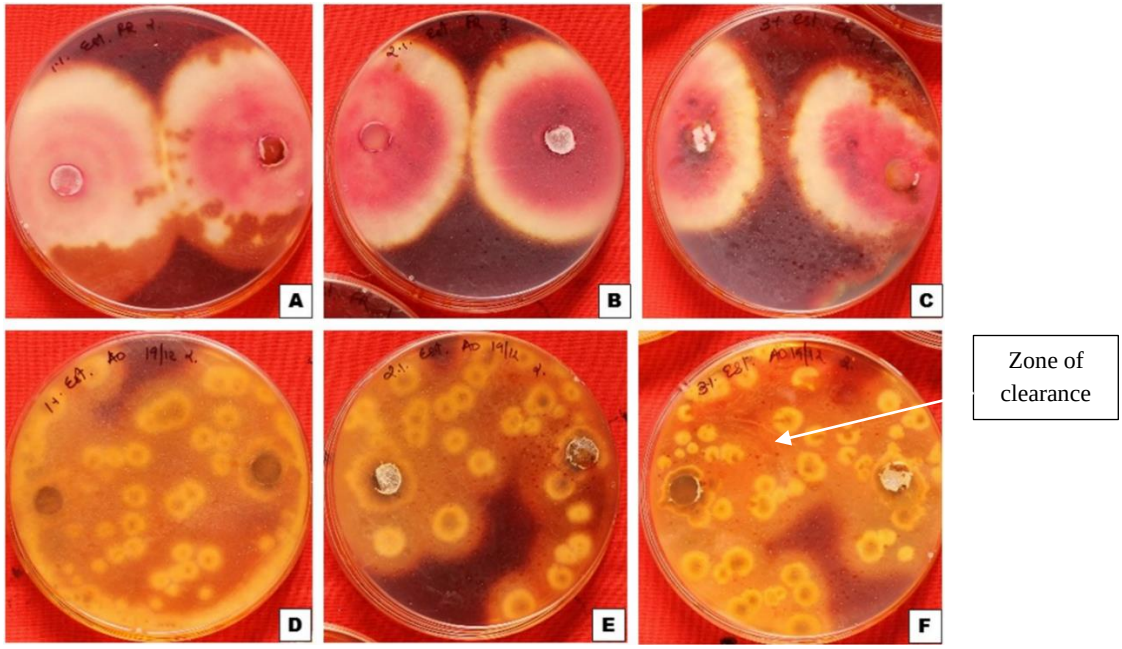

Figure.27. Qualitative analysis of Esterase enzyme activity by fungal strains *F. solani* & *A. oryzae*

A&D: Hydrolysis zone by *F. solani* & *A. oryzae* respectively on media consisting 1% Tributyrin
B&E: Hydrolysis zone by *F. solani* & *A. oryzae* respectively on media consisting 2% Tributyrin
C&F: Hydrolysis zone by *F. solani* & *A. oryzae* respectively on media consisting 3% Tributyrin

Table.12. Result of enzyme plate assays of *F. solani* MN201580.1 (SA17) & *A. oryzae* (SA15)

Fungal Isolates	Proteolytic	Lipolytic	Esterolytic	Ligninolytic
F. solani	Positive	Positive	Positive	Positive
A. oryzae	Positive	Positive	Positive	Positive

Both the strains started producing lipolytic and proteolytic enzymes after fifth day as indicated by the zone of clearance observed. Lipase activity was confirmed by observing fluorescent orange-colored colonies demonstrating the presence of lipase enzyme. Zone of hydrolysis for esterase and ligninolytic enzymes was prominent in *F. solani* MN201580.1 (SA17) culture replicate, while production of lipase enzyme seemed to be faster in *A. oryzae* (SA15) culture plate. Fungal strains had developed prominent halo zones in media containing 1% & 3% tributyrin, displaying the presence of esterase enzyme (Figure.27). Fungal cultures were grown on solid media containing 0.5% skimmed milk powder as source of casein and both the cultures had developed the casein hydrolysis zone (Figure.26-B&E) concluding their ability to produce protease enzymes. Both the fungal species proved to be potential producer of proteolytic, lipolytic and ligninolytic enzymes by developing hydrolysis zone (Table.12).

Evaluation of proteolytic, lipolytic, esterolytic and ligninolytic enzyme activities

A variety of fungal hydrolytic enzymes has been identified and deployed for various purposes in biotechnological studies and yet very few research studies have been performed on the polyethylene degrading capability of fungal hydrolytic enzymes. Therefore, a systematic screening of fungal strains is essential to recognize the key fungal enzymes, with high specific activity and efficacy, involved in polyethylene degradation in particular. Herein, the selected fungal species *F. solani* MN201580.1 (SA17) had been reviewed for their ability to produce such enzymes.

A study of the ability of *F. solani* MN201580.1 (SA17) to produce proteolytic, lipolytic, esterolytic, and ligninolytic as well as hydrolytic enzyme activities in liquid culture media was carried out at various incubation periods (3-35 days) and additionally, for each incubation period fungal biomass was investigated. In each of the three culture broths, a significant increase in biomass was detected as the incubation period increased but gradually biomass concentration decreased reaching the peak as represented in Table.13. The reason behind the decrease is the probably death of fungal mycelium due to depletion of nutrient availability in the media after a certain incubation period.

Laccase production by *F. solani* was investigated and maximum activity was observed on the 35th day (0.57 U/ml) (Wu Yi-Rui et al., 2010). Sowmya et al. (2015) evaluated polyethylene degradation by a *Fusarium* sp., in which laccase production was accounted as 0.00027 ± 0.0001 U/ml amount. In comparison with previous research studies, *F. solani* MN201580.1 (SA17) showed the highest of laccase activity 3.41±0.7 U/ml on the 25th day with (Table.14 & Figure.28-A). The activity gradually

increased from 1.25±0.14 U/ml on 3rd day to 1.86±0.15 U/ml on 15th day of incubation. After fifteen days, it seemed to be drastically increased up to 3.41±0.7 U/ml on 25th day, which again slowly decreased to 2.34±0.2 U/ml laccase activity on 35th day. *F. solani* MN201580.1 (SA17) has shown significant amount of laccase activity when compared to other fungal species.

The MnP enzyme from an unknown species of *Fusarium* was studied and 0.00035 ±0.0001 was recorded on the 5th week of incubation (Sowmya et al. 2015). Another species *Fusarium*, *F. graminearum* has the potentiality to produce manganese peroxidase enzyme inciting the degradation of polyethylene carry bags (Ganesh et al., 2017). Here, *F. solani* MN201580.1 (SA17) exhibited maximum activity as 0.2±0.1 U/ml on the fifteenth day (Table.14 & Figure.28-B), which is higher than other reported *Fusarium* species. The activity showed twofold increase from 0.12±0.02 U/ml on 3rd day to 0.24±0.05 U/ml on 5th day, which again reduced to 0.14±0.03U/ml on 10th day. The activity again showed an increase up to 0.2±0.1 U/ml on 15th day, and again dropped to 0.11±0.02 U/ml in the next incubation period. Surprisingly, the amount gradually risen up to 0.18±0.06 U/ml on 30th and 35th day. The MnP activity was observed to be wavering throughout all incubation periods as represented in Figure.28-B.

Fungal biomass of *F. solani* MN201580.1 (SA17) in malt broth media was calculated to be 0.915±0.01 gm on the twentieth day after inoculation (Table.13 & Figure.28-F). The mycelial growth slowly increased from 0.15±0.02 gm on 3rd day to 0.19±0.03 gm & 0.23±0.04 gm on 10th & 15th day. After that, it showed instant increase up to 0.915±0.01 gm in the next incubation period, which showed instant drop to 0.415±0.02 gm on 20th & 25th day. The maximum mycelial growth can be correlated with the enzyme activity, as the highest growth was noted on 20th day and laccase & MnP activity observed to be maximum on 25th & 15th day of incubation.

Fusarium genera have been recognized as major lipase-producing fungal genera and have been studied for their enzyme activity in PE degradation (Singh and Mukhopadhyay, 2012; Shi K et al., 2020). In different investigations, *F. solani* and *F. oxysporum* had released 0.507 U/ml and 35 U/ml amount of lipase enzyme on the 5th day under agitated conditions (Maia et al., 2001; Rapp, 1995), whereas here in this study *F. solani* MN201580.1 (SA17) had shown 35.09 U/ml lipase activity under stationary condition in the same time period (Table.14 & Figure.28-E). Lipase activity was recorded to be at its peak on the 20th day (51.14±1.76 U/ml) and mycelial growth in the mineral salt medium was likewise seen to be maximum (0.88±0.18 U/ml) around the same time. The activity slowly reached to its peak, 28.95±1.44 U/ml, 35.09±2.06 U/ml, 40.12±2.17 U/ml and 44.32±1.09 U/ml activity recorded on 3rd, 5th, 10th & 15th day, respectively. Lipase enzyme showed slight decrease after maximum activity on 20th day, 44.93±1.31 U/ml and 42.73±0.68 U/ml activity recorded on 30th and 35th day of incubation.

Similar to lipase activity, the esterase enzyme was highest (563.72±2.7 U/ml) on the 20th day and like other activities, it gradually decreased as shown in Table.14 & Figure.28-D. Christakopoulos et al. (1999) reported 7.8 U/ml of esterase activity by *F. oxysporium* after five days. Whereas, in this investigation significant esterase activity was observed by *F. solani* MN201580.1 (SA17). The activity showed drastic increase from 120.5±1.25 U/ml on 3rd day to 178.93±1.50 U/ml and 343.86±2.08 U/ml on 5th & 10th day, respectively, which peaked on 15th day with 559.78±1.62 U/ml activity. A slight decreased activity was recorded in the next incubation period (538.9±1.48 U/ml), which slowly reduced up to 308.45±1.01 U/ml on 35th day.

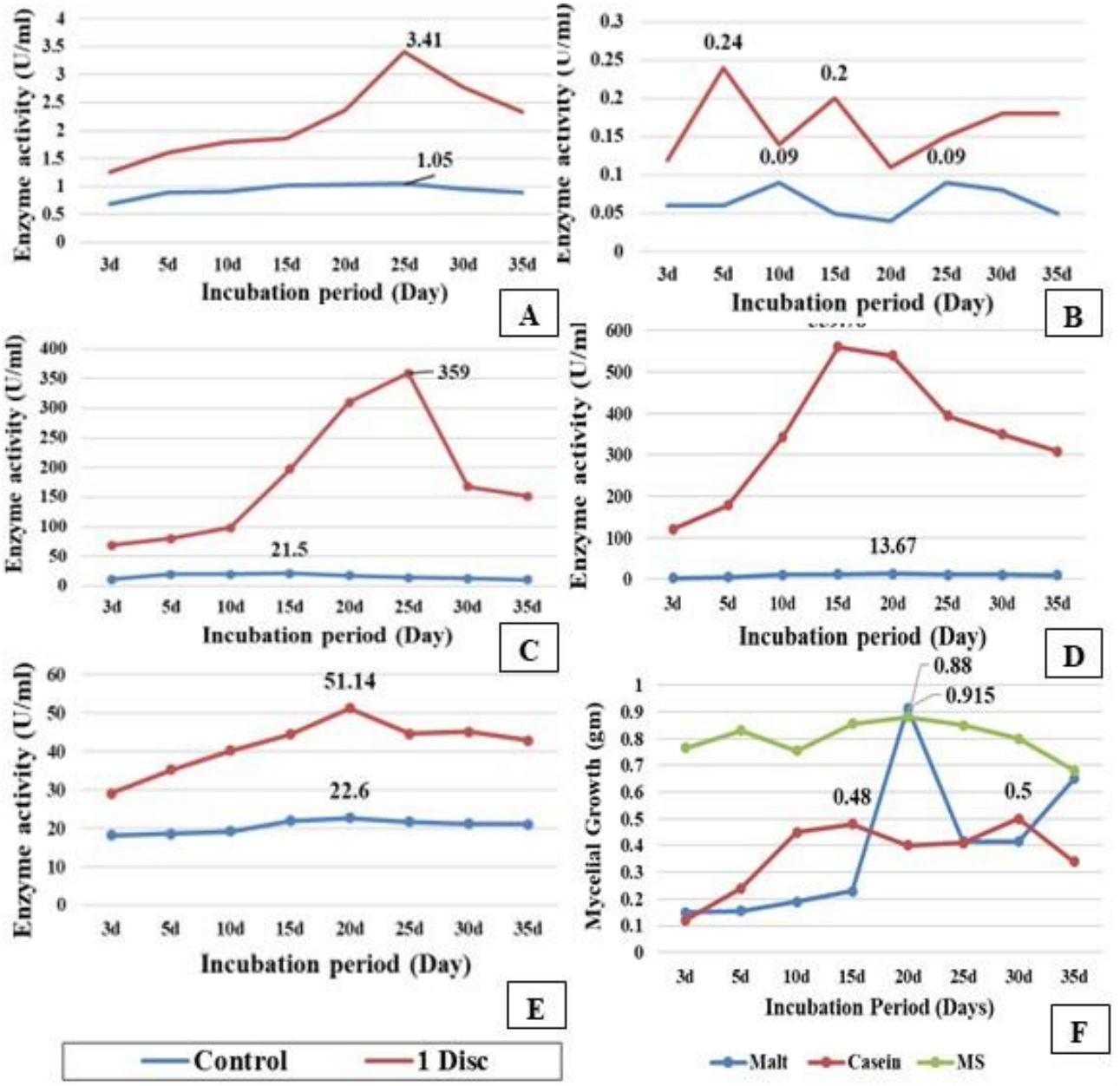

Figure.28. Quantitative analysis of enzyme activities by *F. solani*
A- Laccase activity; **B-** MnP activity; **C-** Protease activity; **D-** Esterase activity; **E-** Lipase activity; **F-** Fungal biomass

The fungal growth in MS broth medium reached a maximum of 0.88±0.18 gm on the 20th day (Table.13 & Figure 22). The biomass exhibited a gradual increase from 0.76±0.06 gm on the 3rd day to 0.85±0.04 gm on the 15th day. However, after reaching the peak growth, the biomass decreased to 0.68±0.08 gm on the 35th day. It is noteworthy that the enzyme activity associated with the maximum mycelial growth, demonstrating a potential relationship between fungal growth and enzymatic activity.

Table.13. Fungal biomass of *F. solani* MN201580.1 produced in culture broths during different incubation period

	Incubation period		**3days**	**5days**	**10days**	**15days**	**20days**	**25days**	**30days**	**35days**
Fungal biomass (gm)	**Lignino-lytic**	**Control**	0.07± 0.02	0.06± 0.005	0.085± 0.02	0.07± 0.01	0.085± 0.01	0.015± 0.005	0.06± 0.06	0.15±0.07
		1 Disc	0.15±0.02	0.155±0.005	0.19±0.03	0.23±0.04	**0.915±0.01**	0.415±0.02	0.415±0.02	0.65±0.05
	Proteo-lytic	**Control**	0.04±0.01	0.13±0.01	0.07±0.01	0.11±0.01	0.07±0.01	0.12±0.02	0.07±0.01	0.08±0.005
		1 Disc	0.12±0.01	0.24±0.005	0.44±0.05	0.47±0.03	0.4±0.005	0.41±0.01	**0.48±0.01**	0.33±0.01
	Estero-lytic & Lipo-lytic	**Control**	0.18±0.08	0.17±0.05	0.18±0.01	0.11±0.03	0.12±0.06	0.12±0.04	0.2±0.03	0.18±0.08
		1 Disc	0.76±0.06	0.83±0.04	0.75±0.15	0.85±0.04	**0.88±0.18**	0.85±0.07	0.8±0.15	0.68±0.08

(Data is statistically significant as p value was <0.05)

Protease enzyme breakdowns the long peptide chain into segmented peptides and *Fusarium* is reported to be a potential fungal species producing protease to degrade plastics (Loredo-Trevino et al., 2011; Cosgrove et al., 2007). Pekkarinen A. et al., found noticeable protease production by *Fusarium* sp. at the point when media glucose had been consumed between the 5th to 7th day of incubation (Pekkarinen et al., 2000). Oppositely, *F. solani* MN201580.1 (SA17) represented maximum (359±2.58 U/ml) protease activity on the 25th day as seen in Figure.28-C & Table.14. The activity gradually increased from 69±5.74 U/ml on third day to 98.5±1.91 U/ml on tenth day, and showed instant increase after that. 197.5±3.41 U/ml and 310.5±3.41 U/ml of enzyme activity was recorded on 10th and 15th day, which peaked on 25th day with 359±2.58 U/ml of activity. After maximum activity, the esterase secretion showed rapid reduction to 167.5±3 U/ml and 151.5±3.41 U/ml on 30th and 35th day, respectively.

Fungal biomass of *F. solani* MN201580.1 (SA17) in casein broth medium was measured during an incubation period. On the 3rd day, the biomass was recorded as 0.12±0.01 gm. Subsequently, it increased to 0.24±0.005 gm on the 5th day and further to 0.44±0.05 gm on the 10th day (as shown in Table.13 and Figure 22). Notably, the mycelial growth remained relatively consistent on the 15th and

30th days, with recorded values of 0.47±0.03 gm and 0.48±0.01 gm, respectively. However, on the 35th day of incubation, there was an abrupt drop in biomass to 0.33±0.01 gm.

Table.14. Enzyme production by *F. solani* MN201580.1 at various incubation periods

	Incubation period		3days	5days	10days	15days	20days	25days	30days	35days
Enzyme activity (U/ml)	Laccase	Control	0.68 ± 0.04	0.9± 0.05	0.91 ± 0.07	1.02 ± 0.07	1.04 ± 0.07	1.05 ± 0.08	0.96 ± 0.02	0.89 ± 0.06
		1 Disc	1.25±0.14	1.6±0.08	1.8±0.11	1.86±0.15	2.37±0.08	**3.41±0.7**	2.78±0.05	2.34±0.2
	MnP	Control	0.05±0.01	0.06±0.01	0.09±0.01	0.05±0.01	0.04±0.01	0.09±0.02	0.08±0.01	0.05±0.01
		1 Disc	0.12±0.02	0.24±0.05	0.14±0.03	**0.2±0.1**	0.11±0.02	0.15±0.09	0.18±0.09	0.18±0.06
	Protease	Control	11±3	20±1.41	20±3.65	21.5±2.51	18±1.63	14±1.63	13±2.58	10.5±1.91
		1 Disc	69±5.74	80±3.65	98.5±1.91	197.5±3.41	310.5±3.41	**359±2.58**	167.5±3	151.5±3.41
	Esterase	Control	3.06±0.48	5.106±0.88	10.83±1.04	12.53±0.99	13.67±0.66	11.46±0.59	11.23±0.74	9.92±0.39
		1 Disc	120.5±1.25	178.93±1.50	343.86±2.08	**559.78±1.62**	538.9±1.48	393.95±1.13	348.73±1.13	308.45±1.01
	Lipase	Control	18.09±0.69	18.45±0.86	19.13±0.32	21.8±2.87	22.6±3.1	21.57±0.43	21.06±0.59	21.01±0.62
		1 Disc	28.95±1.44	35.09±2.06	40.12±2.17	44.32±1.09	**51.14±1.76**	44.47±1.23	44.93±1.31	42.73±0.68

(Data is statistically significant as p value was <0.05)

Overall, in this experiment, *F. solani* MN201580.1 (SA17) and *A. oryzae* (SA15) were evaluated for their enzyme activities, including laccase, MnP, esterase, lipase, and protease. Among these two strains, *F. solani* MN201580.1 (SA17) demonstrated a higher potential for producing enzymes that can break down complex structures into smaller segments. Based on the qualitative analysis of fungal enzymes, SA17 unveiled a more prominent zone of clearance compared to SA15. As a result, only SA17 was selected for quantitative analysis. Ligninolytic enzymes, specifically laccase and MnP, reached to its peak activity on the 25th and 15th day, respectively, with values of 3.41±0.7 U/ml and 0.2±0.1 U/ml. Lipolytic enzymes, including lipase and esterase, showed the maximum activity on the 20th day (51.14±1.76 U/ml) and 15th day (559.78±1.62 U/ml), respectively. Proteolytic activity, on the other hand, was recorded to be the highest on the 25th day (359±2.58 U/ml) for the SA17 fungal strain. The result indicates that the SA17 fungal strain exhibits a significant capacity to secrete enzymes responsible for the breakdown of polyethylene.

Key observations of the study

The responsible enzymes, Laccase, MnP, Protease, Esterase & Lipase were evaluated qualitatively and quantitatively.

- Qualitative assay revealed potentiality of *F. solani* MN201580.1 (SA17) to release these enzymes by showing zone of hydrolysis around the colonies.
- In the quantitative analysis, *F. solani* MN201580.1 (SA17) showed significant amount of enzyme activities indicating its ability to breakdown polyethylene material.