Twenty four soil samples were collected from different agricultural fields of Ibrahimabad, Medak district, Andhra Pradesh, India, cultivating sweet sorghum and many other monocot crops. All the fungal isolates were screened for ferulolyl esterases in two phases. Phase 1 consisted of a plate assay method and phase 2 as submerged cultures. The fungal isolates were screened on a minimal agar medium containing (g l^{-1}): $(NH_4)_2SO_4$ 1.5, KH_2PO_4 0.5, $MgSO_4$ 0.25, $CaCl_2$ 0.05, $FeCl_3$ 0.01, yeast extract 1.0 and bacteriological agar 20.0. The sterile medium was supplemented with ethyl ferulate (1%) in 5% solution of dimethylformamide (DMF). A total of about 150 fungi were isolated from these 24 soil samples and further screened for their ability to produce feruloyl esterases

Forty two feruloyl esterase producing fungi were isolated based on this screening procedure. The cultural and morphological characterization of these 42 isolates indicated that they belonged to 6 different morphotypes after dereplication. Two were only identified to the genus level: *Aspergillus* sp. and *Sclerotium* sp. The remaining fungi, identified up to the species were *Aspergillus terreus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Lasiodiplodia theobromae*. *Aspergillus terreus*, *Lasiodiplodia theobromae* and *Sclerotium* sp., are the three new species which were new additions to the list of feruloyl esterase-producing fungi.

The HPLC protocol was standardized for quick detection and quantification of both ferulic acid (product) and methyl ferulate (substrate) with retention times of 3.9 and 8.57 minutes, respectively. The feruloyl esterase activity in the culture supernatants of the liquid minimal medium was found to range between 0.05 to 6.7 U ml^{-1}. Among these 42 isolates, the feruloyl esterase activity of *Aspergillus terreus* was high (6.7 U ml^{-1}), while the production ability of *Lasiodiplodia theobromae* and *Sclerotium* sp. was very low (1.27 and 0.96 U ml^{-1}, respectively). *Aspergillus terreus* GA2 identified as a promising feruloyl esterase producer was selected for further studies on optimization of feruloyl esterase production, characterization and application of ferulic acid recovery from maize bran as a lost-cost lignocellulosic susbtrate.

In order to obtain maximum yields of feruloyl esterase, solid state fermentation (SSF) conditions for enzyme production were standardized. Effective feruloyl esterase production was observed with maize bran as substrate followed by wheat bran, coconut husk and rice husk among the tested agro-waste crop residues. Optimum particle size of 0.71-0.3 mm and moisture content of 80% favored the enzyme production. Moreover, optimum feruloyl esterase production was observed at pH 6.0 and a temperature of 30°C. Supplementation of potato starch (0.6%) and casein (1%) as as carbon and nitrogen sources, respectively, favored enzyme production. Furthermore, the culture produced the enzyme after 7 days of incubation when the C:N ratio was 5. Optimization of the SSF conditions revealed that maximum enzyme activity (1162 U gds^{-1}) was observed after 7 days in a production medium of 80% moisture content and pH 6.0 containing maize bran 16 g (25%, w/v) of particle size of 0.71-0.3 mm, potato starch 0.6%, casein 3.0% and 64 ml of formulated basal salt solution [(per liter): $(NH_4)_2SO_4$ (1.5 g), KH_2PO_4 (0.5 g), $MgSO_4$ (0.25 g), $CaCl_2$ (0.05 g), $FeCl_3$ (0.01 g), Na_2HPO_4 (1.5 g)]. Overall, the enzyme production was enhanced by 3.2 fold as compared to the un-optimized conditions.

Facile and efficient chemical synthesis methodologies were developed to prepare the esters of acids and alcohols. The synthesis of these different aliphatic, aromatic saturated and unsaturated esters is based on Lewis acid catalyzed esterification (Compounds 1-11). The synthesis of aryl ester is based on the coupling of acid and phenol by DCC (Compound 12).The synthesis of aliphatic esters is based on the coupling of acid and diazomethane (Compound 13). The synthesis of different aryl acetates is based on esterification by acetic anhydride (Compounds 15-18).The synthesis of monoacetate from meso-diol was done enzymatically using porcine pancreatic lipase (PPL) (Compound 19). The compounds were synthesized to characterize and to calculate the catalytic efficiencies of the three purified FAEs. Hydrolytic profiling was done on the basis of (a) Changes in types and substitution on the benzene ring (substrates 1-11), (b) changing of the aliphatic region substitution group on ester bond (substrates 2, 4 and 12), (c) position and number of ester bond on benzene ring (substrates 13-18), and (d) requirement of benzene ring for ester bond cleavage (substrate 19).

The enzymes from *Aspergillus terreus* strain GA2 responsible for the feruloyl esterase activity were purified to homogeneity using ion-exchange, hydrophobic interaction and gel filtration chromatographies, and designated as AtFae-1, AtFae-2 and AtFae-3. Biochemical characterization and classification of these enzymes were done. The enzymes were monomeric in nature having molecular masses of 74 kDa, 23 kDa and 36 kDa. Active proteins bands were identified by a developed pH-dependent zymogram on native PAGE. The three enzymes also exhibited variation in pH tolerance ranging between pH 5-8 and temperature stability of up to 55°C. Inhibition studies revealed that the serine residue is essential for the FAE activity; moreover aspartyl and glutamyl residues were not totally involved at the active site of the enzymes. Metal ions such as Ca^{2+}, K^{+} and Mg^{2+} increased and stabilized the enzyme activity for all the three feruloyl esterases. Kinetic data indicated that all three enzymes showed good catalytic efficiencies (k_{cat}/K_m) against different synthesized alkyl and aryl esters indicating their broad substrate specificity. The peptide mass fingerprinting by MALDI/TOF-MS analysis and enzyme affinity towards methoxy and hydroxy substituents on the benzene ring revealed that the AtFae-1 was type A and AtFae-2, AtFae-3 were type C feruloyl esterases. Moreover our studies on the role of different feruloyl esterases on various types of substrates revealed that these enzymes not only acted on cinnamic acid esters but also on alkyl and aryl esters. This suggests their importance in not only releasing ferulic and/or cinnamic acids but also different alkyl/aryl compounds like phenolic acids from plant material.

The *Aspergillus terreus* strain GA2 under study produced multiple enzymes can thus acted synergistically in releasing ferulic acid from agro-waste substrates. Taguchi L-9 Orthogonal Array was adopted to study the impact of different parameters for the release of ferulic acid from maize bran by treating it with extracellular FAE and other hydrolytic enzymes from the crude enzyme extract of *Aspergillus terreus* GA2. Three factors were optimized using Taguchi statistical design, among them temperature contributed the maximum impact [45%] on the overall ferulic acid yield followed by the enzyme dosage [43%]. pH showed the least impact [8%] at the individual level. When these levels were applied in the experiment to release ferulic acid, there was 8% increase in the yield which was 13.8 mg gds^{-1} as compared to the conventional method of optimization. 70% of the alkali extractable ferulic acid was obtained on enzyme treatment

and 86% ferulic acid was recovered using powdered activated charcoal. >95% pure ferulic acid was obtained after silica gel chromatography which was confirmed by ^{1}H NMR and ESI mass spectroscopy. After optimization of the enzymatic hydrolysis conditions for recovery of ferulic acid, this method was scaled up and the ferulic acid yield obtained was 14.2 g kg^{-1} of dry maize bran. This indicated that a significant amount of ferulic acid was released from maize bran using the crude extract of *Aspergillus terreus* GA2. The recovery and purification method developed was efficient.

Considering the different salient properties and their catalytic efficiency for different substrates, the feruloyl esterases from *Aspergillus terreus* GA2 can be attractive candidates for different biotechnological applications.