International Journal of Basic and Applied Biology
Print ISSN: 2394-5820, Online ISSN: 2349-2539, Volume 2, Number 4; January-March, 2015, pp. 258-261

http://www.krishisanskriti.org/ijbab.html

Microbial Release of Ferulic Acid Esterase from Agro-residue using *Trichophyton ajelloi* MTCC 4878

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Abstract—*Phenolic moieties, which include Ferulic acid and p-coumaric acid, are linked to polysaccharides in the plant cell wall by ester or ether bond forming structural cross-linking bridges. The wall bound ferulic acid can be released by cleaving the ester bond using enzyme called Feruloyl esterase, also known as Ferulic acid esterase, thus releasing the ferulic acid into the medium. Ferulic acid production was observed with rice bran as substrate followed by wheat bran, rice husk and sugarcane bagasse among the tested agricultural residues. In current study, an ability to produce FA was checked for Trichophyton ajelloi by inoculating the microbe in media containing agricultural residues. Supplementation of agricultural residue as carbon source and soya bean meal as nitrogen source favoured ferulic acid esterase production, enzyme responsible for release of Ferulic acid. Moreover, optimum Ferulic acid esterase production was observed at pH 6 and temperature 30°C.*

1. INTRODUCTION

Biological degradation of the accumulated agricultural, industrial, organic as well as toxic wastes has become the most popular alternative for various economic and ecological reasons and has led to the possibility of extracting valuable products to reduce the cost of treatment and disposal. A high proportion of this waste material includes carbohydrate and phenolic groups. Phenolic moieties, which includes ferulic acid and p-coumaric acid, are linked to polysaccharides in the plant cell wall by ester or ether bond forming structural cross-linking bridges [12].

Ferulic acid is the most abundant hydroxycinnamic acid in the plant kingdom and occurs mainly in the cell wall of cereal plants, which is covalently linked to lignin with ether bonds [9]. Ferulic acid esterases (FAE's, also known as Feruloyl esterases cinnamoyl esterases or cinnamic acid esterases; EC: 3.1.1.71) that represent a diverse group of esterases can release ferulic acid form plant cell wall constituents. Ferulic acid esterases are key enzymes for cell wall hydrolysis and are used for the extraction of phenolic acids from agricultural residues [6, 8]. Additionally ferulic acid esterases, have the ability to release caffeic acid, p-coumaric acid and ferulic acid from agro-industrial by products like coffee pulp, apple marc and wheat straw [2]. Ferulic acid esterases were first identified in *Streptomyces viridosporus* [3]; later they were purified and characterized from various fungi like *Aspergillus niger* [1], *Aspergillus flavipes* [10], *Aspergillus awamori* [5] and some bacteria such as *Staphylococcus aureus* [12], *Lactobacillus acidophilus K1* [13].

Feruloyl esterases are used for processing agricultural residues into valuable products. Based on the microbial degradation of ferulic acid into vanillin [7] and also its antioxidant properties, a number of industrial and food applications have been reported. Ferulic acid acts as a potential chemo preventive agent for AIDS by inhibiting AIDS virus [4]. It is said to have cholesterol-lowering activity [11].

This work represents the capability of *Trichophyton ajelloi* MTCC 4878 to release ferulic acid esterase inoculated in minimal media containing different agricultural residues as carbon source supporting the growth of microbe.

2. MATERIAL AND METHODS

2.1 Chemicals

The microbiological media and other medium ingredients like ammonium sulphate, soya bean meal, potassium di-hydrogen phosphate, magnesium sulphate, disodium hydrogen phosphate, ferric chloride and calcium chloride were procured from HI Media Laboratories Pvt. Ltd., Mumbai, India. Ethyl ferulate (98%), ferulic acid (99%) and methanol were procured from Sigma Aldrich (HPLC grade).

2.2 Fungal strain and culture conditions

Fungal culture i.e. *Trichophyton ajelloi* MTCC 4878 was obtained from the departmental stock (Department of Bio-engineering, Birla institute of technology, Mesra, Ranchi). The isolates were revived in fresh slants of Sabouraud dextrose agar and incubated for a period for 6 days. A loop full of

fungal isolate was transferred into Potato dextrose broth and incubated at room temperature for 6 days until a green film develops. The broth containing the fungal growth is placed in the refrigerator for further use.

2.3 Agro-waste processing and fermentation conditions

Finely grounded agricultural residue was obtained from rice mill, wheat mill. Minimal media containing (per litre) NH_4SO_2 (1.5g), KH_2PO_4 (.5g), $MgSO_4$ (.25g), $CaCl_2$ (.05g), $FeCl_3$ (.01g), Soya bean meal (1g), Na_2HPO_4 (1.5g) was prepared in distilled in water. 25ml of this medium was transferred in several 100 ml flask and autoclaved for 20 minutes at 121°C. The autoclaved medium was inoculated with the fungal isolate and agro- residue and incubated at 37°C for an interval of 4-10 days, under static conditions.

2.4 Crude enzyme production and activity analysis

The agricultural residue and the fungal biomass was separated from the medium containing enzyme using filter paper after 4-12 days of incubation. Quantification of enzyme activity was done by HPLC using ethyl ferulate as a substrate.

HPLC reaction was prepared using 0.1ml of ethyl ferulate and 0.5ml of crude enzyme incubated at 37°C water bath for 30 minutes. The reaction was stopped by adding equal volume of methanol: acetic acid in the ratio of 4:1. The reaction mixture was then filtered using 0.2 µm syringe filter. HPLC analysis was done on a reverse phase HPLC equipped with a C_{18} column (Waters XTerra RP18 5µm, 4.6x150). HPLC set up includes a waters 2996 photodiode array detector, waters 717 plus auto sampler and waters 1525 binary HPLC pump. The result was analysed using Empower pro software. A sample volume of 20 µl was injected and the product (ferulic acid) and substrate (ethyl ferulate) was eluted in an isocratic solvent system of water: methanol (40:60 ratio) as the mobile phase at the flow rate of 1ml/ min for 8 minutes. The peak of product and substrate was observed at 254 to 310 nm. Ferulic acid esterase activity is calculated from the ferulic acid standard curve.

2.5 Optimization of production condition

The initial experiments were conducted to evaluate the influence of different agricultural residues viz. rice bran, wheat bran, sugarcane bagasse and rice husk on the release of ferulic acid esterase. These agricultural residues were used as carbon source. Soya bean meal was used as nitrogen source, as optimized by Sachan *et al., 2014,* to enhance the production of ferulic acid esterase.

2.6 Optimization of environmental factors (concentration of agro- residue, days, pH and temperature)

Firstly the effect of days was optimized by incubating minimal media with fungal isolates containing different agricultural residues for a period of 4-12 days and the activity of enzyme was assayed through HPLC using ethyl ferulate as substrate. Once the agro-residue resulting in maximum enzyme activity with minimum incubation period was optimized, it was followed by studying the effect of concentration, ranging from 2%- 10%. Optimization of pH and temperature for maximum enzyme activity was carried out by incubating the media for 4-12 days at a range of 25- 45°C and a pH ranging from 4- 10. Similarly for the effect of concentration, pH and temperature was evaluated using ethyl ferulate as a substrate. HPLC analysis and Bradford's assay (for protein concentration) of crude extract was done to calculate the specific activity of the enzyme.

3. RESULTS AND DISCUSSION

3.1 Optimization of agricultural residue and incubation period

In our studies, submerged fermentation conditions were optimized for the production of ferulic acid esterase using *Trichophyton ajelloi* MTCC 4878 for different agricultural residues. Agricultural residue acted as a sole source of carbon and soya bean meal was used as nitrogen source. The incubation period ranged from 4- 12 days at 30- 37 °C and pH 5. As a result of which rice bran was observed to show the maximum specific activity on the 6th day incubation. However comparatively low activity was observed on 4th, 8th and 10th day of incubation (Fig. 1).

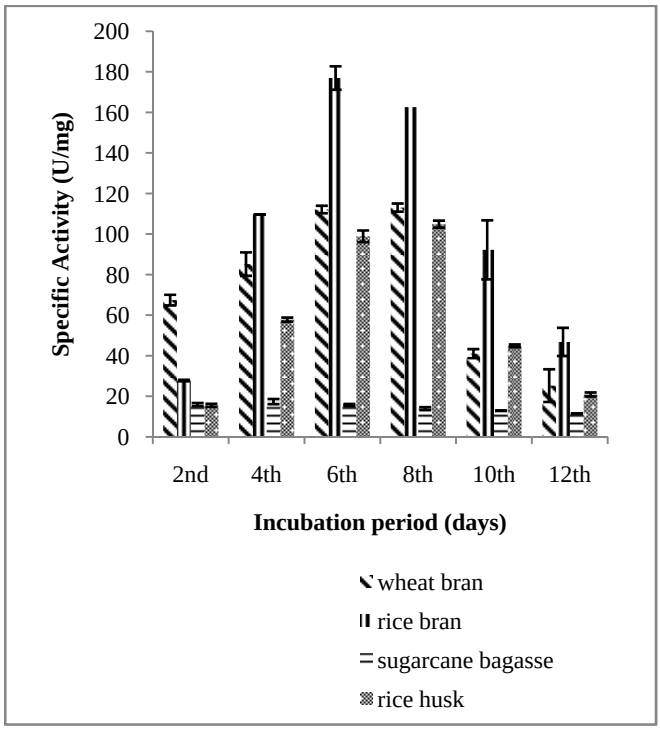

Fig. 1: Effect of agricultural residue and incubation period on the release ferulic acid esterase by *Trichophyton ajelloi* MTCC 4878

3.2 Optimization of concentration of agro- residue

Different concentrations of rice bran ranging from 2- 10% were evaluated for maximum enzyme activity using ethyl ferulate as substrate. The culture was incubated for 6 days at 30- 37°C and pH 5 (Fig. 2). At 4- 6% maximum enzyme activity was observed with decrease in activity at 8 and 10%. The optimal concentration of agricultural residue was optimized as 4%.

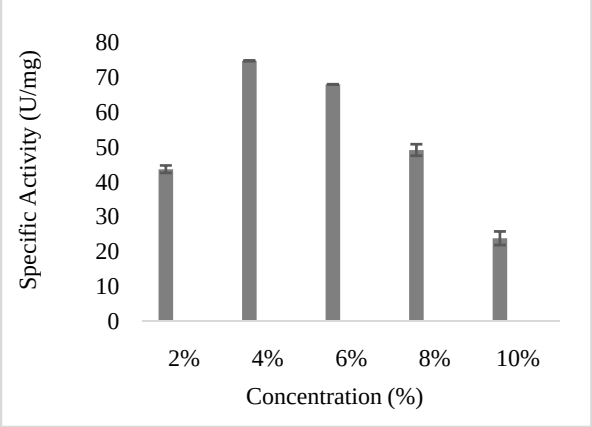

Fig. 2: Effect of different concentrations of rice bran on ferulic acid esterase production

3.3 Optimization of pH

The optimal pH was determined by measuring the FAE activity at different pH ranging from 4- 10 where the maximum activity was observed at pH 6.0 (Fig. 3). There was significant enzyme production in the pH range of 5- 7, however the specific activity of enzyme was very low above pH 7, indicating that alkaline conditions does not support enzyme production from *Trichophyton ajelloi* MTCC 4878.

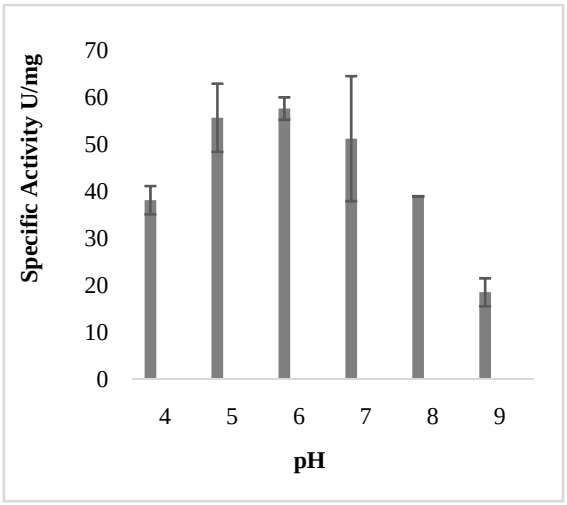

Fig. 3: Effect of pH on ferulic acid esterase production

3.4 Optimization of temperature

Temperature optimization for production ferulic acid esterase was performed using 1% ethyl ferulate as substrate and pH of the medium was kept as 5. The temperature ranging from 25-45°C was evaluated. Maximum enzyme production was observed in temperature range of 25- 30 °C where 30 °C was observed as optimum temperature for the production of FAE from *Trichophyton ajelloi* MTCC 4878 (Fig. 4). The enzyme activity decreased significantly at 35 °C and above which may be due to the loss in enzyme activity because of degradation of enzyme's secondary structure at high temperature Sachan *et al.*, 2014.

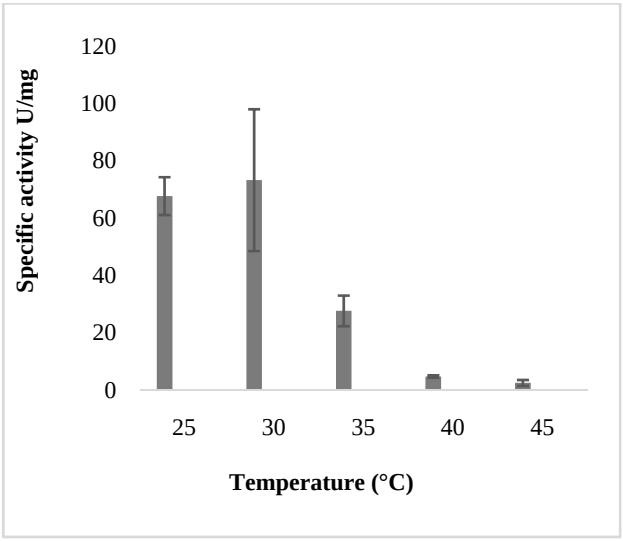

Fig. 4: Effect of temperature on the release of ferulic acid esterase

4. CONCLUSION

The present results showed that the fungus *Trichophyton ajelloi* MTCC 4878 was able to grow under submerged fermentation culture conditions using an easily available agricultural residue i.e. rice bran for the production of ferulic acid esterase. The agro- residues used in this study have shown their importance as carbon source for ferulic acid esterase production. Purification of ferulic acid from agricultural residues is in progress.

5. ACKNOWLEDGEMENT

Authors acknowledge DST-SERB, GOI Reg. No. SR/FT/LS-46/2012 for providing financial support for consumables and Attika Gami is thankful to MHRD, GOI for providing GATE scholarship. We are also thankful to Centre of Excellence (COE) Ref No. NPIU/TEQUIP II/FIN/31/158, dated April 16, 2013 and Department of Bio-engineering, BIT Mesra, Ranchi for providing the lab infrastructure.

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