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Enhanced Production of Ferulic Acid Esterase by Microorganism

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Abstract—*The enzyme ferulic acid esterase or feruloyl esterase is a member of enzyme family hydrolases, specifically those acting on carboxylic ester bond. Ferulic acid, a phenolic, phytochemical, is the largest among the other hydroxycinnamic acid to be found in the plant cell wall. The useful hydrocinnamic acid such as ferulic acid and p-coumaric acid, present in the plant cell wall, are linked to the polysaccharide with the ether bond or the ester bond. This enzyme holds its importance mostly because it can release 3-methoxy-4-hydroxy cinnamic acid or ferulic acid which is used as antioxidant and the precursor for various commercially important molecules, vanillin is one of them. The important and the cheapest method for the production of fruloyl esterase is its microbial production Various microorganisms has been reported toproduce ferulic acid esterase. The present work is focussing on enhanced production of FAE by Trichophyton ajelloi with the use of optimised parametric conditions. Supplimentation of glucose and tryptic soya was favourable for the production of Ferulic acid esterase. It was observed in the study that the optimum production of ferulic acid esterase was at temperature 300^{0}C and the pH 6.*

Keywords: *ferulic acid, ferulic acid esterase, microorganism.*

1. INTRODUCTION

Phenolic acids are integral stuctural components of the plant cell wall, as they have the capacity of cross-linking the polysaccharide chains through a dimerisation reaction.Ferulic acid (FA), the most abundant hydroxycinnamic acid in the plant kingdom, covalently linked via ether bonds to lignin are found mainly in the cell wall of cereal plants[4]. It is the precursor of coniferyl alcohol and is found conjugated with mono- and oligosaccharides, polyamines, lipids and polysaccharides and can sometimes occur in a free state.

Ferulic acid (FA) was first isolated and purified from the plant *Ferula foetida* in 1866. Later this compound was confirmed as 3-methoxy-4-hydroxycinnamic acid. It is a phenyl propenoid . Ferulic acid exists as a crystalline solid.

It is a phenolic phytochemical present in large amount in the plant cell wall components like arabinoxylans as covalent side chain. FA is present in the plant cell wall in form of cis and trans isomer (cis, yellow oily liquid; trans, crystalline structure).

The systematic name of the enzyme feruloyl esterase is 4-hydroxy-3-methoxycinnamoyl-sugar hydrolase.. Ferulic Acid Esterases (FAEs; EC.3.1.1.73; also known as feruloyl esterase, cinnamic acid hydrolases, cinnamoyl esterases) are subtypes of carboxylic acid esterases. FAEs act on the ester bond in the plant cell wall which is present between sugar and any phenolic compound. These enzymes hydrolyze the ester bond and release the hydroxycinnamic acid that was bound with the polysaccharides[2].

FAEs have a wide range of applications in various types of industries. FAEs finds its application in industries such as paper, biofuel, medical, food, and cosmetic industries. In the paper and pulp industry, FAEs are used to remove the fine particles from pulp[5]. As there is a drastic increment in the demand for methanol, there FAEs also gain importance in the biofuel industries. Since FAEs have a wide range of biotechnological importance, these enzymes are being studied from a large number of microorganisms i.e. bacteria and fungi.

In this study ferulic acid esterase production has been studied under optimized growth conditions for improved levels of ferulic acid esterase from a strain *Trichophyton ajelloi* MTCC4878.

2. MATERIALS AND METHODS

2.1 Chemicals

The microbiological media and other media ingredients like ammonium sulphate,soya bean meal, potassium di-hydrogen phosphate, magnesium sulphate, disodium ,hydrogen phosphate, ferric chloride, calcium chloride, yeast extract and tryptic soya were procured from HI Media Laboratories Private Limited, Mumbai, India Ethyl ferulate (98%), ferulic acid(99%) and methanol were procured from Sigma Aldrich (HPLC grade). Bradford’s protein estimation kit was purchased from Merck Specialities Private Limited,Mumbai, India. All the other chemicals used in this study were of analytical grade.

2.2 Microorganism and culture condition

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 culture was revived in fresh slants of Sabouraud dextrose agar and incubated for a period for 6 days at room temperature.

Since the fungal culture taken was spore forming culture, the starter culture was prepared by taking a loopful of the culture and streaking it into the already prepared potato dextrose agar slants. The were then incubated at 28-30^{0}C for a period of six days.

2.3 Ferulic Acid esterase enzyme production

The fungal culture slants were taken and the fungal spores were dissolved in distilled water. The starter culture (1x10^{8}spores/ml) was transferred into eight 100ml Erlenmeyer flask containing 25ml minimal media. The pH of the medium was maintained at 6-6.5 and the media was supplemented with 5mM ethyl ferulate (before inoculation of the culture). The inoculated flasks were kept in incubator at 30^{0}C for 8 days. The media was withdrawn periodically for the analysis of FAE activity.

2.3 Analysis of Ferulic Acid Esterase activity

At regular interval of 24 hours, one flask is withdrawn each day till the eighth day of incubation. The fungal biomass was filtered out using filter paper followed by centrifugation. Quantification of enzyme activity was done by HPLC using ethyl ferulate as a substrate.

The reaction mixture was prepared by mixing 200 µl enzyme extract , 400 µl substrate ethyl ferulate (1mg/ml dissolved in dimethylformamide) , ATP and $MgCl_2$ added in ratio of 1:2. The reaction mixture was incubated at the room temperature for different time intervals. The reaction was stopped by adding 600 µl methanol and acetic acid in the ratio 1:4 then the mixture was filtered using syringe fiulter. The HPLC analysis was carried out using Waters column (XTerra, C-14 5 µm, 4.6 × 150 mm Column). The analysis of result was done using the Empower Pro software. The sample volume was 20 µl and the substrate (EF) along with the product (FA) was eluted in an isocratic solvent system of Milli-Q and 100% methanol in the ratio of 68:32 in the mobile phase at a flow rate of 1 ml/min for 15 mins and monitored at 310 nm. Ferulic acid esterase activity is calculated from the ferulic acid standard curve.

2.4 Optimisation of conditions producing the enzyme

The intial study was done to evaluate the effect of static condition and agitation on the production of the enzyme. The effect of adding glucose as supplement to the culture media was also studied. Soya bean meal was used as nitrogen source, as optimized by Sachan *et al., 2014,* to enhance the production of ferulic acid esterase.

2.5 Optimisation of the environmental parameters

At first the incubation period was optimized by adding the fugal culture in the medium for a period of 4-10 days. The activity of enzyme was assayed through HPLC using ethyl ferulate as substrate.

Optimization of pH and temperature for maximum enzyme activity was done by incubating the media for 4-10 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 DISCUSSIONS

3.1 Comparison of the production of ferulic acid esterase in static and shaking condition

Agitation maintains the proper aeration in the medium. Less agitation would result in improper aeration, thus less growth of microorganisms whereas more agitation may result in microbial cell disruption. Thus, it is necessary to optimize shaking condition for appropriate growth of microbial cells. Here, a comparative study has been done on the production of the FAE by *Trichophyton ajelloi* MTCC 4878 at shaking condition and at static condition. Shaking condition with 100 rpm was maintained for the study and it was observed to be suitable for product formation in case of *Trichophyton ajelloi* MTCC 4878 as compared to the static condition.

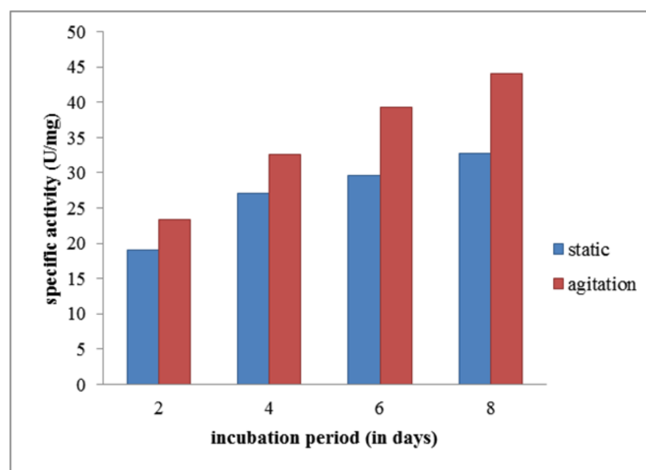

Fig. 1: Comparison of the production ferulic acid esterase in static and shaking condition by *Trichophyton ajelloi* MTCC 4878.

3.2 Study of the effect of glucose on the production ferulic acid esterase

Carbon source (glucose) helps to form of high density culture resulting in reduced time period with increase in the

concentration of product formation(Oddou *et al.* ,1999) . Here, a comparative study was done on the production of FAE by using glucose as sole carbon source and the production of FAE by not using any carbon source other than the substrate. 0.1% (w/v) glucose was added to the minimal media for the growth of the organism and when all the glucose was utilized by the organism them substrate was added to the medium.The production of FAE by *Trichophyton ajelloi* MTCC 4878 was more in the glucose supplemented media. The maximum enzyme activity of 236.36 U/mg was observed on the 8th day of incubation in the glucose supplemented media.

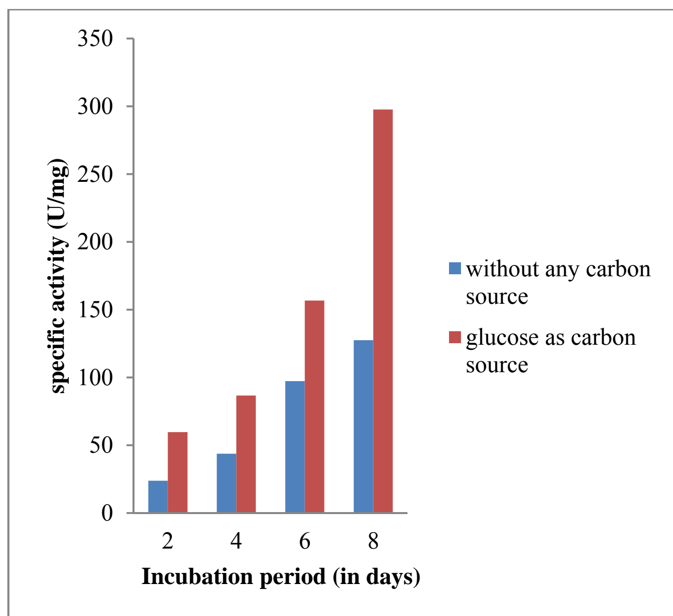

Fig. 2: Study of the production ferulic acid esterase by using glucose as carbon source by *Trichophyton ajelloi* MTCC 4878

3.3 Optimization of pH

The opmisation of pH was carried out by measuring the FAE activity at different pH ranging from 4.5-7.5. The maximum enzyme activity was observed at pH 6.5 (Fig. 3). There was significant enzyme production in the pH range of 5.5- 7, but the specific activity of enzyme was very low above pH 7. This shows that alkaline conditions does not support enzyme production from *Trichophyton ajelloi* MTCC 4878.

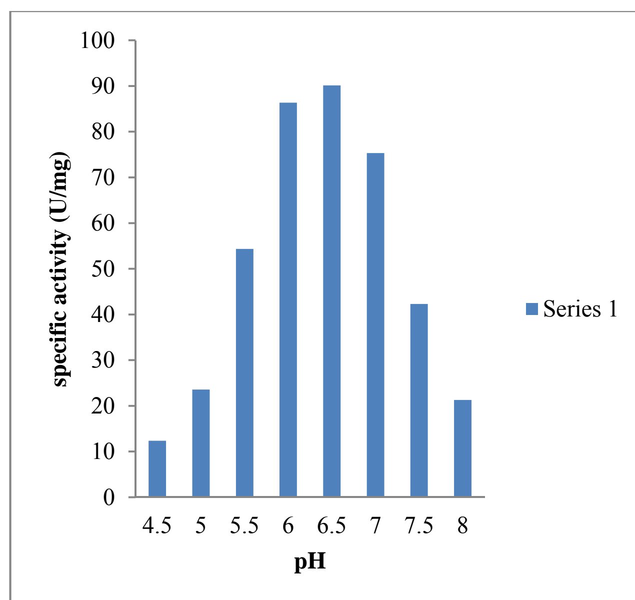

Fig. 3: Effect of different pH on the production of ferulic acid esterase by *Trichophyton ajelloi* MTCC 4878.

3.4 Optimization of temperature

Temperature optimization for production ferulic acid esterase was carried out using 1% ethyl ferulate as substrate and pH of the medium was kept as 5. The effect of ferulic acid esterase production at temperature ranging from 25-45°C was studied. 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. The enzyme activity was observed to decrease significantly at the temperature above 35 °C which may be due to the loss in enzyme activity because of degradation of enzyme's secondary structure at high temperature.

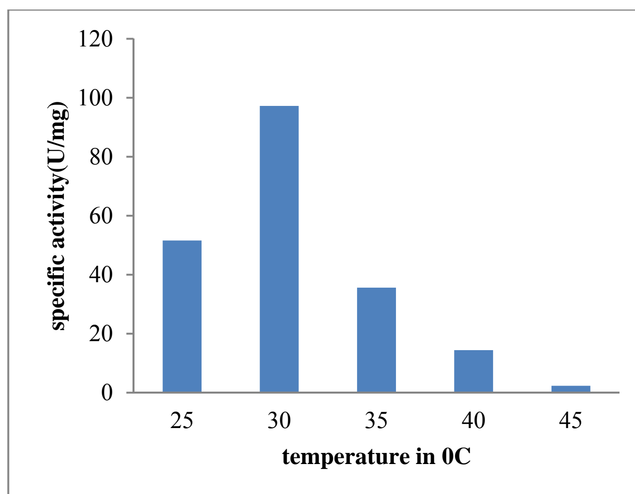

Fig. 4: Effect of temperature on the release of ferulic acid esterase by *Trichophyton ajelloi* MTCC 4878.

4. CONCLUSION

From the result of the present study it can be concluded that the production of ferulic acid esterase by *Trichophyton ajelloi* MTCC 4878 is maximum with glucose as supplement at pH 6.5 , temperature 30^0C and at 100rpm shaking condition.

5. ACKNOWLEDGEMENT

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