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Bioconversion of Maize Straw into Ferulic Acid by *Pseudomonas fragi* MTCC 10212

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Abstract: Isolation of ferulic acid from maize straw was carried out using *Pseudomonas fragi* MTCC 10212 by the action of ferulic acid esterase. Ferulic acid was detected as the important phenolic acid from the medium. The compound was identified and quantified by high performance thin layer chromatography. Ferulic acid was detected for a period of 10 days. Maximum quantity of the acid was quantified on 7 day of incubation and the quantity declined on further incubation. Concentration of carbohydrates from the destarched straw was also determined and was compared with that of original straw. Approximately 260 mg of ferulic acid was obtained per Kg of maize straw on 7 day of incubation. *Pseudomonas fragi* was solely responsible for the extraction of ferulic acid from maize straw which is an essential phytochemical with antioxidant and antimicrobial properties. It can be easily bioconverted into vanillic acid, salicyclic acid, vanillin, etc. which are useful raw materials in Pharmaceutical, food and flavouring industries.

Key words: *Pseudomonas fragi*, ferulic acid, maize straw, HPTLC.

Introduction

Ferulic acid (FA) chemically known as 4-hydroxy-3-methoxycinnamic acid is a ubiquitous phenolic compound in plant tissues. It is the most abundant hydroxycinnamic acid widely found in vegetables, fruits and some beverages such as coffee and beer [4]. Though the compound has been reported to posses many physiological functions such as antimicrobial, anti-inflammatory, anti-thrombosis anti-cancer activities, etc [12], recently this phenolic acid has gained attention for its potential role as an adjuvant therapy for several free radical-induced diseases. FA was proposed as a novel antioxidant compound with a strong cytoprotective activity due to both the ability to scavenge free radicals and activate cell stress response [14]. FA plays an essential role in providing the rigidity to the cell wall and formation of other important organic compounds like coniferyl alcohol, vanillin, sinapic, diferulic acid and curcumin [12]. Ferulic acid, being highly abundant, may be functional as a precursor in the manufacturing of vanillin, a synthetic flavoring agent often used in place of natural vanilla extract [11]. Several *in vivo* and *in vitro* studies have been conducted in humans, animals and cell culture [1,9,13,15] to establish the function of FA.

In plants, FA is relatively abundant in cell walls. The compound is usually concentrated in the bran of grains, peel of fruits, and roots and peel of vegetables. It is one of the metabolites of lignin biosynthesis and is found both in free and conjugated form in plants [21]. These are found covalently linked to polysaccharides by ester bonds and to

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components of lignin by ester or ether bonds. FA represents up to 1.5 % dry weight of cell wall materials in the members of *Poaceae* family. The lignin component of this lignocellulosic biomass acts as the polyphenolic macromolecule from which the phenolic acid such as ferulic acid can be isolated [3]. Ferulic acid esterase (FAEs EC 3.1.1.73) being a subclass of the carboxylic acid esterases plays a central role in degradation of intricate structure of the plant cell wall by cleavage of ester bonds between hydroxycinnamic acids esterfied to arabinoxylans (AXs) and certain pectins present in plant cell walls [19]. Various studies were conducted regarding the action of FAE by using different microorganisms for production of ferulic acid [2,6,10,22]. Varieties of crop residues those are rich in lignocellulosic biomass could act as a renewable resource for the extraction of FA. The production of bioenergy and chemicals from agricultural waste and agro-industrial residues may somewhat change the concept more towards the production of biofuel and chemical rather than food use [20]. Maize straw is one such crop residue that can be used as an alternate source of phenolic acids as these are abundantly found in nature and are very effective [7]. In this paper, we have focused at the production ferulic acid from maize straw by the action of *Pseudomonas fragi* MTCC 10212. The process optimization for the maximum product recovery has also been studied.

Materials and methods

Microorganism and cultural condition

Pseudomonas fragi MTCC 10212 was obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh. The microorganism was maintained on the nutrient agar medium. The cultures were incubated at 30°C for 7 day for next subculture.

Crop residues (Maize straw)

Maize straw samples were obtained from common maize (*Zea mays*) cultivars grown in farm of Orissa University of Agriculture and Technology, Bhubaneswar, India. The maize straw in form of long dried leaves left in the field after harvesting was used as the samples for this study. Samples were stored at -4°C for pretreatment process.

Pretreatment of samples

The maize straws obtained from field were cut manually into small pieces of 1 cm length and were grinded into powder having mesh size 0.211 mm. Before using as substrates, maize straw was destarched by following the modified method of Johnson *et al.* [8]. The straw powder was treated with 0.50 % (w/v) potassium acetate at 80°C for 45 min and was then washed several times with tap water. Then straw was recovered by filtration and was soaked into filter paper and dried overnight inside the hot air oven. The dried pretreated maize powder was autoclaved for 20 min at 121°C to inactivate any endogenous enzymes and proteinaceous inhibitors and stored at -20°C.
Estimation of total sugar release

Total sugar was estimated by phenol sulphuric acid method [5] using glucose as standard. 1.0 ml of filtered solution of destarched and non-destarched maize straw was taken in test tubes. To this solution 1.0 ml phenol solution was added and mixed well. Then 5.0 ml of conc. H_2SO_4 was added to the mixture and left for 30 minutes. The absorbance was recorded at 488 nm and total sugar was determined from the standard curve.

Fermentation

The microorganism was grown in minimal medium having ammonium nitrate (3.0 g l^{-1}), hydrated magnesium sulphate (0.2 g l^{-1}), sodium chloride (0.2 g l^{-1}), potassium dihydrogen phosphate (1.0 g l^{-1}), disodium hydrogen phosphate (4.0 g l^{-1}) and calcium chloride (0.05 g l^{-1}). The initial pH of the minimal medium was adjusted to 7.0 before autoclaving for 15 min at 121°C and the cultures were incubated at 30°C. The pretreated processed maize straw was used as the substrate for the isolation of ferulic acid. 1 ml of inoculum was used in each flask containing substrate and incubated till 10 days for analysis on day by day basis.

Analysis and detection of ferulic acid

Culture supernatants were prepared by filtration process. These were acidified (pH 1-2) and extracted with equal volume of ethyl acetate. The

ethyl acetate was evaporated using the rotary vacuum evaporator and residue was dissolved in 50% methanol and used for high performance thin layer chromatography. Separation and identification of ferulic acid was performed by using high performance thin layer chromatography having wavelength UV-detector (CAMAG Linomat 5). Samples are sprayed onto HPTLC plates (034.5642 HPTLC plates Merck Silica gel 60 F254, 20 x 10 cm in size) in the form of bands with nitrogen as carrier gas. The identification of ferulic acid in sample was confirmed by comparing retention times of standard ferulic acid. The quantification of ferulic acid was done by the comparing the peak areas using win CATS software

Statistical analysis

All the measurements were carried out at least three times and were presented as the arithmetic mean ± standard deviation (SD).

Results

The carbohydrate content of both de-starched and control samples were quantified (Fig. 1). The carbohydrate content of de-starched and control sample was 560 µg ml^{-1} and 1785 µg ml^{-1} respectively. *Pseudomonas fragi* was inoculated in minimal medium containing de-starched maize straw and incubated for 10 days at 30°C. Quantification of FA was performed by the HPLTC for 10 d. Results revealed that the amount of ferulic acid increased continuously up to 7 days of incubation. The maximum amount of FA detected on 7 days of incubation was 260 mg kg^{-1} of straw. The amount of ferulic acid when measured declined after 7 days of incubation and was very negligible on 10 days of incubation (Fig. 2). During the course of biodegradation process, along with ferulic acid another metabolite was also detected in the HPTLC chromatograph (Fig. 3).

Discussion

Over the past years, consumer demand for natural ingredients has widely increased. It has been established that natural flavours include products that are obtained through microbial or enzymatic processes as long as the precursor or raw material is of natural origin. Based on the above concept, the biotechnological production of vanilla flavour also requires a suitable starting compound. Thus, considering the alternative natural sources of ingredients as substrates for production processes based on microbial biotransformation, have become an increasingly attractive option. Due to chemical similarity between ferulic acid and vanillin, the biotransformation of ferulic acid to vanillin is a study of interest. In order to produce "natural vanillin", it is necessary to use "natural ferulic acid" i.e. released from raw materials by GRAS (Generally Regarded As Safe) enzymes.

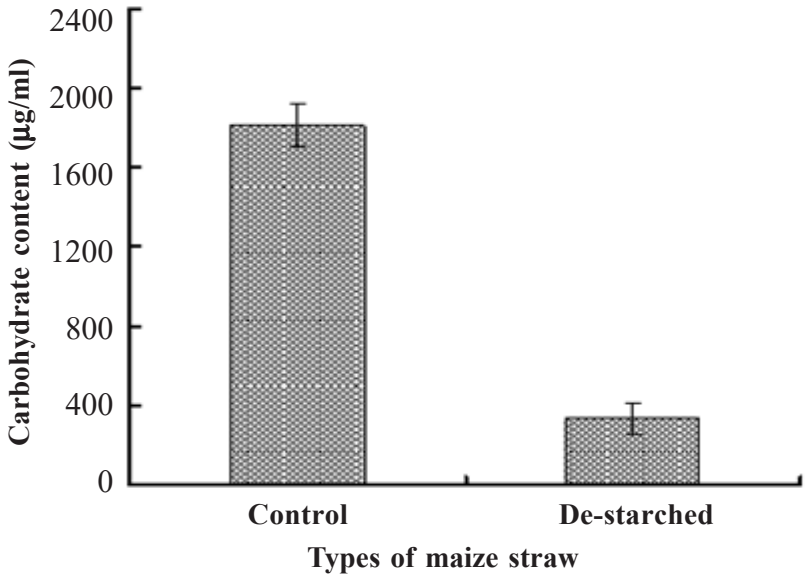

Fig. 1. Carbohydrate contents of maize straw

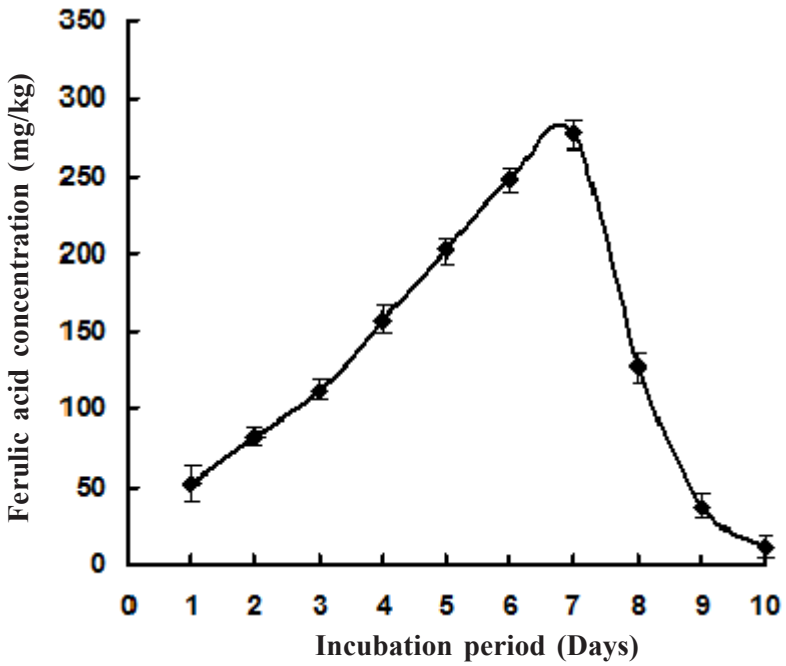

Fig. 2. Concentration of FA from maize straw by *Pseudomonas fragi* MTCC 10212

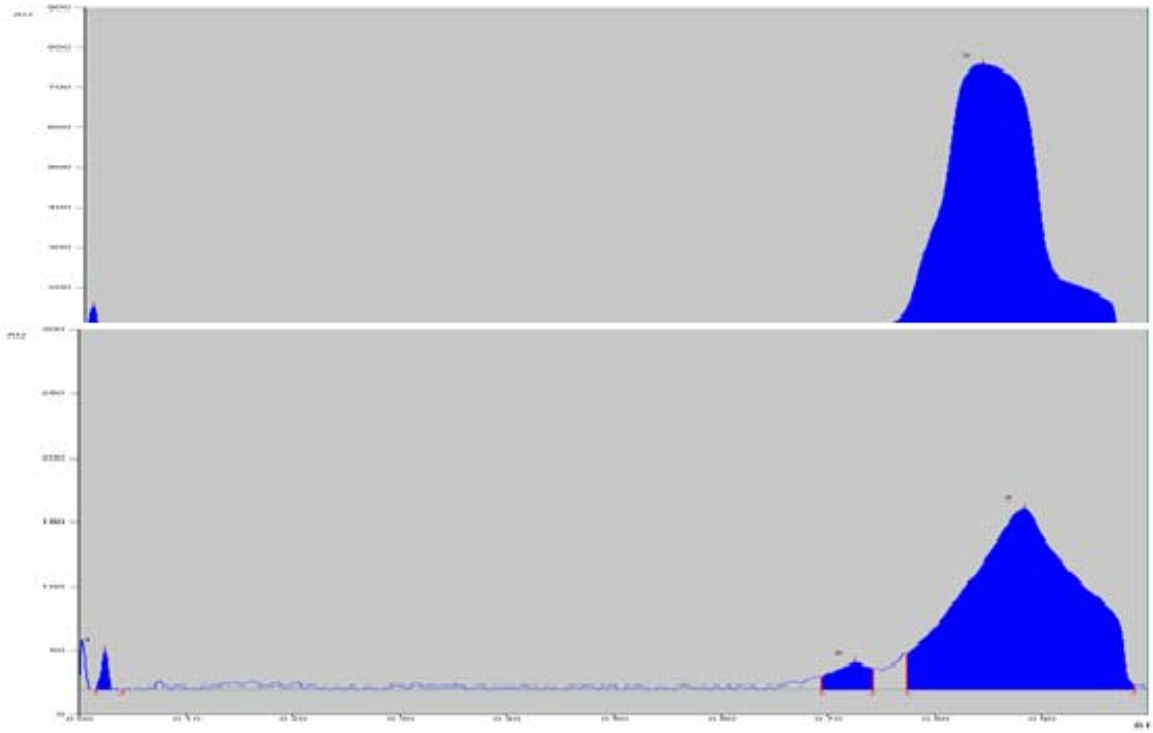

Fig. 3. HPTLC chromatogram of ferulic acid. (Upper) represents the standard FA and (Lower) represents the released FA from maize straw

Many species of microorganisms have shown their ability to transform or degrade the above stated plant aromatic compounds [16]. Filamentous fungi can be an important source of phenol-degrading species as they grow frequently in wood where phenolic structures are present. Strains of bacteria, yeast and other fungi have also shown to metabolize hydroxycinnamic acids to their cor-

responding hydroxybenzoates. Hence, due to the abundance of these natural aromatic products, much scientific interest has been focused on the ability of microorganisms to metabolize phenolic compounds such as hydroxycinnamic acids, in order to discover novel systems for the formation of phenolic flavouring and aromas. Therefore, it can be proposed that the concept of natural biodegradation has led to the evolution of biotransformation technologies, which are important tools for renewing natural resources by conversion into commercially valuable products.

Ferulic acid is the ubiquitous phenolic compounds and constitutes the bioactive ingredient of many foods. Though FA is naturally found in many parts of the plants its extraction from the various residues like straw and husk of many crop plants through biotransformation needs to be emphasized. In the present study, maize straw is being used as source of FA. Maize straw is a byproduct and represents an abundant but underexploited renewable resource. Special attention is focused on the extraction of anti-oxidants from these inexpensive sources by microbial degradation. Enzymatic upgrading of straw is an attractive alternative to environmentally damaging chemical methods currently used for lignocellulose saccharification. Over the last decade, concentration on the isolation and purification of number of microbial esterase which can cleave FA from sugar residues in agro-industrial waste was emphasized. In this case study, maximum amount of FA (260 mg kg^{-1}) was obtained on 7 d day of incubation using *P. fragi* MTCC 10212. Our previous study on FA different *Streptomyces* isolates (S 39, LM 30 and S 10) confirmed about the amount of FA obtained by these *Streptomyces* isolates were 213 mg, 155 mg and 64 mg per Kg of wheat bran respectively [17]. These studies indicate that crop residues including maize straw could be used as best sources for isolation of FA for a variety of industrial applications. *Staphylococcus aureus* was selected for the isolation of ferulic acid from wheat bran [18]. Amount of isolated ferulic acid was continuously increases from 1st day to 7th day of incubation. It was confirmed that about 275 mg of ferulic acid was obtained from 1kg of wheat bran after 6th days of incubation period. It was confirmed that the ferulic acid esterases activities of *Staphylococcus aureus* was higher than the ferulic acid esterases activities of different *Streptomyces sp.* The enzyme that catalyzes the conversion of process optimization and the enzymatic activities of various other microorganisms may further enhance the quantity of FA extraction that needs to be further investigated. Also the other metabolite that was detected along with FA could be some other phenolic compounds like vanillin or vanillic acid that are the degradation products of FA.

Conclusion

Pseudomonas fragi was selected for the isolation of ferulic acid from maize starw. A efficient HPTLC method has been developed to separate hydroxycinnamic acids and their hydroxybenzoates derivatives. Maximum amount of FA (260 mg kg-1) was obtained on 7 day of incubation using this microorganism. It was seen that, the product accumulation remained stable for a considerably long period of time. Therefore, *Pseudomonas fragi* might be of significance from the point of view that, this strain could be useful as ferulic yielding strains leading to the improvement of production of vanillate derivatives. This strain could also be competently shuffled with some strains of hyper producing ferulic acid esterase activity if mutant enzymes could be evolved or imported from other genomes and used in the pathway of improved strain, resulted from genome shuffling.

References

1. **Balasubashini, M.S., Rukkumani, R., Viswanathan, P., Menon, V.P. (2004).** Ferulic acid alleviates lipid peroxidation in diabetic rats. Phytother. Res. 18: 310-314.
2. **Converti, A., Aliakbarian, B., Domínguez, J.M., Bustos Vázquez, G., Perego, P. (2010).** Microbial production of biovanillin. Braz. J. Microbiol. 41(3): 519-530.
3. **Cruz, J.M., Dominguez, J.M., Dominguez, H., Parajo, J.C. (2001).** Antioxidant and anti-

microbial effects of extracts from hydrolysates of lignocellulosic materials. J. Agric. Food Chem. 49: 2459-2464.

4. **D'Archivio, M., Filesi, U., Di Benedetto, R., Gargiulo, R., Giovannini, C., Masella, R. (2007).** Polyphenols, dietary sources and bioavailability. Ann. Ist Super Sanita. 43:348-361.
5. **Dubois, M., Giles, K.A., Hamilton, J.K., Robers, P.A., Smith, F. (1956).** Colorimertic method for determination of sugars and related substances. Anal. Chem. 28: 350-356.
6. **Gami, A., Singh, S., Sachan, A. (2015).** Microbial Release of Ferulic Acid Esterase from Agro-residue using *Trichophyton ajelloi* MTCC 4878. Int. J. Basic Appl. Biol. 2(4): 258-261.
7. **Gonzalez, J., Cruz, J.M., DomInguez, H., Parajo, J.C. (2004).** Production of antioxidants from *Eucalyptus globulus* wood by solvent extraction of hemicellulose hydrolysates Food Chem. 84: 243-251.
8. **Johnson, K.G., Harrison, B.A., Schneider, H., Mackenzie, C.R., Fontana, J.D. (1988).** Xylan-hydrolysing enzymes from *Streptomyces* spp. Enzyme Microbiol Technol. 10: 403-409.
9. **Kampa, M., Alexaki, V.I., Notas, G., Nifli, A.P., Nistikaki, A., Hatzoglou, A., Bakogeorgou, E. (2004).** Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: Potential mechanisms of action. Breast Cancer Res. 6: 63-74.
10. **Kaur, B., Chakraborty, D., Kumar, B. (2013).** Phenolic Biotransformations during Conversion of Ferulic Acid to Vanillin by Lactic Acid Bacteria. BioMed Res. Int. 6 pages.
11. **Kawsar, S.M.A., Huq, E., Nahar, N., Ozeki, Y. (2008).** Identification and Quantification of Phenolic Acids in *Macrotyloma uniflorum* by Reversed Phase-HPLC. American Journal of Plant Physiology. 3(4): 165-172.
12. **Kumar, N. and Pruthi, V. (2014).** Potential applications of ferulic acid from natural sources. Biotechnology Reports. 4: 86-93.
13. **Lin, F.H., Lin, J.Y., Gupta, R.D., Tournas, J.A., Burch, J.A., Selim, M.A., Monteiro-Riviere, N.A., Grichnik, J.M., Zielinski, J., Pinnell, S.R. (2005).** Ferulic acid stabilizes a solution of vitamins C and E and doubles its photoprotection of skin. J. Invest. Dermatol. 125: 826-833.
14. **Mancuso, C., Santangelo, R. (2014).** Ferulic acid: Pharmacological and toxicological aspects. Food Chem. Toxicol. 65: 185-195.
15. **Ogiwara, T., Satoh, K., Kadoma, Y., Murakami, Y., Unten, S., Atsumi, T., Sakagami, H. (2002).** Radical scavenging activity and cytotoxicity of ferulic acid. Anticancer Res. 22: 2711-2717.
16. **Rosazza, J. (1995).** Biocatalysis, microbiology and chemistry: the power of positive linking. ASM News. 61: 241-245.
17. **Sarangi, P.K., Sahoo, H.P., Ghosh, S., Mitra, A.P. (2009).** Isolation of ferulic acid from wheat bran using *Streptomyces* isolates. Int. J. Appl. Agri. Res. 4: 57-62.
18. **Sarangi, P.K, and Sahoo, H.P. (2010).** Ferulic Acid production from wheat bran using *Staphylococcus aureus*. New York Sci. 3(4): 79-81.
19. **Tarbouriech, N., Prates, J.A., Fontes, C.M., Davies, G.J. (2005).** Molecular determinants of substrate specificity in the feruloyl esterase module of xylanase 10B from *Clostridium thermocellum*. Acta. Crystallogr. D Biol Crystallogr. 61: 194-197.
20. **Zhang, Y.H.P. (2008).** Reviving the carbohydrate economy via multi-product lignocelluloses bioefineries. J. Ind. Microbiol. Biotechnol. 35: 367-375.
21. **Zhao, Z., Moghadasian, M.H. (2008).** Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. Food Chem. 109: 691-702.
22. **Zheng, L., Zheng, P., Sun, Z., Bai, Y., Wang, J., Guo, X. (2007).** Production of vanillin from waste residue of rice bran oil by *Aspergillus niger* and *Pycnoporus cinnabarinus*. Bioresour. Technol. 98: 1115-1119.