



**PS-026**

## **EXTRACTION OF FERULIC ACID FROM AGRO-INDUSTRIAL WASTES AND EVALUATION OF BIOCONVERSION OF FERULIC ACID TO VANILLIN BY *STREPTOMYCES SETONII***

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### **Abstract**

Several agro-industrial wastes (as chestnut and pistachio shells, grass, leaf fruit, vine leaf, and, red and white grape stems) were evaluated to ferulic acid extraction. The chemical analysis of these raw materials shows a high content of xylan in pistachio shells (33%), following the biorefinery concept, this fraction can be used in xylitol manufacture. The lignocellulosic materials were submitted to an alkaline extraction to obtain phenolic compounds, reaching a concentration of 312 mg ferulic acid/L in grass wastes. Other phenolic compounds were also extracted (gallic acid, p-coumaric acid, syringic acid etc.) depending of used raw material. Besides, the ferulic fermentation into vanillin was assayed. The higher concentration of vanillin (2692 mg/L) was achieved at 27 h ( $Q_p = 99.704 \text{ mg/L}\cdot\text{h}$  and  $Y_{p/S} = 0.50 \text{ mg/mg}$ ) using *Streptomyces setonii*.

*Keywords*: Ferulic acid, agro-industrial wastes, vanillin, *Streptomyces setonii*

### **INTRODUCTION**

Similar to petroleum, lignocellulosic materials (LCMs) have a complex composition and, as such, their fractionation in hemicelluloses, celluloses and lignin allows obtaining a wide range of products (FitzPatrick et al., 2010). In biorefineries, added value products such as

ferulic acid (FA) extracted from LCMs represents an important component of the overall value chain and economics (Abokitse et al., 2010).

FA can be released by chemical and enzymatic hydrolysis from LCMs. In chemical treatments is common to use non-pressurised and pressurised alkaline hydrolysis, although FA can also be released by other pressurized solvents (ethanol, water and ammonia) (Buranov and Mazza, 2009), meanwhile enzymatic hydrolysis is carried out by feruloyl esterases (Topakas et al., 2007).

FA can also be metabolized in other value added products as 4-vinylguaiacol (VG) and vanillin (V) (Mathew et al., 2007). The commercial cost of VG is nearly 40 times higher than that of FA (Mathew et al., 2007). Nowadays, consumers have increased the demand of natural flavors, since they have natural and healthy attributes (Hua et al., 2007). Thus, interest of natural V production by biotechnological processes has grown.

V can be produced by *Streptomyces setonii* (Muheim and Lerch, 1999). In its metabolic pathway, V is an intermediate metabolite of the microbial degradation of FA (Muheim and Lerch, 1999). V is further oxidized to vanillic acid, which is either O-demethylated to protocatechuic acid or decarboxylated to guaiacol (Priefert et al., 2001)

The aim of this work was to study the extraction of ferulic acid from different agro-industrial waste and evaluation of media composition for bioconversion of synthetic FA by *S. setonii*.

## **METHODOLOGY**

### **Raw materials**

Seven LCMs with local agricultural and processing origin were considered in this work: chestnut and pistachio shells, grass, leaf fruit, vine leaf, and, red and white grape stems. All materials were dried, milled to a particle size less than 1 mm, homogenized in single lots to avoid compositional differences, distributed to smaller containers, and stored at low humidity conditions at room temperature.

### **Alkaline hydrolysis of pre-treated materials**

The treatment was carried out according to Salgado et al. (2012).

### **Microorganism, medium composition and cultures conditions**

*Streptomyces setonii* CECT 3276 was purchased to Spanish Type Culture Collection (Valencia, Spain) equivalent to *Streptomyces setonii* ATCC 39116 and maintained in cryovials at -40 °C using 30% glycerol as cryoprotector. Inocula were prepared by dissolving the previous glycerol stock cryovials in 250 mL Erlenmeyer flasks containing 50 mL of the following basal medium: 10 g commercial glucose/L, 4 g yeast extract/L, 4 g Na<sub>2</sub>HPO<sub>4</sub>/L, 1 g KH<sub>2</sub>PO<sub>4</sub>/L, 0.2 g MgSO<sub>4</sub>•7H<sub>2</sub>O/L, 0.2 g NaCl/L and 0.05 g CaCl<sub>2</sub>•H<sub>2</sub>O/L, and pH adjusted to 7.2 with a solution of NaOH 2M. The preculture was grown overnight before inoculation, 2 mL of grown medium were added to other Erlenmeyer flask with basal medium. After glucose was consumed 5 g/L of FA was added to medium. FA dissolution was sterilized by ultrafiltration using 0.22 µm membranes (Nalgene). Samples of 1 mL were recovered along of fermentation in sterile conditions. Samples were centrifuged and analyzed by HPLC. All assays were performed in duplicate.

### **Analytical determinations**

The treatment was carried out according to Salgado et al. (2012).

## **RESULTS**

### **Characterization of raw materials**

Seven raw materials (chestnut and pistachio shells, grass, leaf fruit, vine leaf, and, red and white grape stems) were characterized by quantitative acid hydrolysis (Salgado et al., 2012). Table 1 shows the obtained results.

Pistachio shells had a higher content in hemicelluloses, of which xylan showed a 33.1%. This fraction can be hydrolyzed to obtain liquors with high concentration of xylose and can

be fermented to xylitol by *Debaryomyces hansenii* (Salgado et al., 2012). Cellulose changes widely from only 10.1 % in vine leaf to 22.2 % in grass.

	Cellulose	Hemicelluloses			Lignin	Other compounds	
		Total	Xylan	Arabinan			Acetyl groups
Chestnut shells	21.1	13.9	10.5	1.4	2.0	46.5	18.6
Leaf fruit	11.1	14.7	9.2	1.2	4.3	20.5	53.8
Pistachio shells	15.2	38.5	33.1	0.0	5.4	29.4	17.0
Red grape stem	13.3	8.47	6.7	0.77	1.0	35.9	42.4
Switchgrass	22.2	13.9	10.4	3.5	0.0	18.0	46.0
Vine leaf	10.1	8.37	5.9	1.8	0.67	44.4	37.1
White grape stem	20.7	11.37	10.4	0.00	0.97	31.3	36.7

**Table 1. Quantitative acid hydrolysis (in %) of agro-industrial waste (Salgado et al. 2012).**

### Ferulic acid extraction

Different phenolic compounds and concentrations were released depending on the material employed: gallic acid (628.0 mg/L in chestnut shells), p-coumaric acid (542.7 mg/L in grass), p-hydroxybenzoic acid (394.5 mg/L in leaf fruit), ferulic acid (312.0 mg/L in grass), vanillic acid (192.7 mg/L in vine leaf), syringic acid (116.2 mg/L in pistachio shells), and 3,4-dihydroxybenzaldehyde (66.8 mg/L in chestnut shells). Vanillin and vanillic alcohol were detected in negligible amounts (< 0.5 mg/L) with the exception of the small amounts (17.3 mg/L) quantified in white grape stem.

### Evaluation of bioconversion of ferulic acid in vanillin

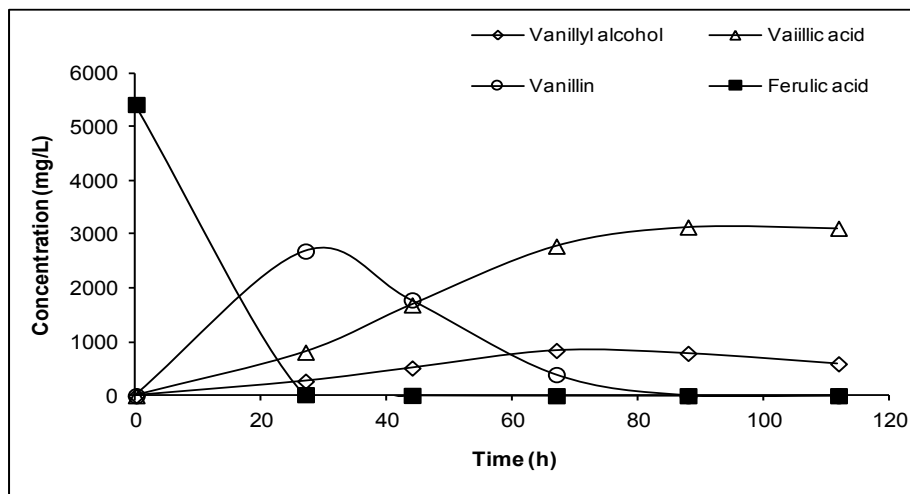
Previously to the use of FA obtained by hydrolysis of LCMs, synthetic FA was assayed to study the V production by *S. setonii*. Once the microorganism was grown in a medium basal with glucose, FA was added after glucose consumption. Fig. 1 shows the production of vanillin, vanillic acid and vanillyl alcohol after FA addition. FA was quickly degraded to vanillin, producing a maximum of 2692 mg/L of vanillin after 27 h of fermentation (99.704

mg/L·h and 0.50 mg/mg). Higher fermentation times result in a degradation of V to produce 3139 mg/L vanillic acid after 88h.

Among the materials considered in this work, grass showed the highest concentration of ferulic acid (Table 2).

Pre-treated materials	Ferulic acid (mg/L)
White grape stem	97.9
Red grape stem	92.2
Vine leaf	35.8
Leaf fruit	64.0
Grass	312.0
Pistachio shells	43.0
Chestnut shells	19.5

**Table 2. Ferulic acid released from pre-treated materials (Salgado et al., 2012).**



**Fig. 1. Bioconversion of ferulic acid in vanillin by *S. setonii***



## CONCLUSIONS

Agro-industrial wastes can be a source of FA. The released of FA was integrated in fractionation stages of LCMs. From all these wastes, grass showed the highest concentration of FA. *S. setonii* can metabolize synthetic FA to vanillin in basal medium. The following works will focus on the bioconversion of FA present in hydrolyzates to V. Different treatments of FA purification from LCMs will be necessary to improve productivities and yields.

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