Enhancing the rate of ferulic acid bioconversion utilizing glucose as carbon source

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Abstract: Work has been carried out to study the effect of glucose addition into the medium during the biotransformation of ferulic acid into vanillin using *Staphylococcus aureus*. Study showed that microorganism consumed ferulic acid very quickly having more than 4-fold accumulation of vanillin (45.7 mg/l) on 2nd day as compared to 9.8 mg/ml of vanillin accumulation on 7th day without addition of glucose. [Journal of American Science 2010; 6(5):115-117]. (ISSN: 1545-1003).

Key words: biotransformation, ferulic acid, *Staphylococcus aureus,* glucose, vanillin

1. Introduction

Hydroxycinnamic acids such as ferulic acid and *p*-coumaric acid occur widely in the cell walls of graminaceous plants (Grabber et al 1995, Harris and Hartley 1980). Ferulic acid is a very important component for the structure and the biology of cell wall as it can cross link polysaccharide chains through dimerisation reaction as described by Ishii (1997). Microbes transform hydroxycinnamic acids to their corresponding hydroxybenzoates upon the production of ferulic acid esterase (FAE) enzyme. FAE has the greater industrial importance like lipases having microbial origin (Jooyandeh et al 2009). These benzoates are important components of natural flavours and fragrances. Like the antioxidant activities of some plants extracts as described by Jayaprakasha et al (2008), hydroxycinnamates can also act as precursors for a variety of antioxidant compounds, signaling molecules and phytoalexins that play an important role in plant defense responses (Dixon et al 1995). A number of industrial and food applications were reported for ferulic acid, especially based on its microbial degradation to vanillin. Vanillin is the world's most highly prized natural flavour. It is one of the most important aromatic flavour compounds used in foods, beverages, perfumes and pharmaceuticals (Clark 1990)**.** Thus, considering the increasing interest in 'natural' products, the production of flavours via biotechnological processes offers a viable alternative to natural and chemical sources (Walton et al 2003). This work reports the capability of *Staphylococcus aureus* to degrade ferulic acid into vanillin. Effect of carbon sources on the production of metabolites was a study of interest (Vijayendra et al 2008). In this case study the effect of supplementation of glucose into the medium was analyzed during the biotransformation of ferulic acid into vanillin.

2. Materials and Methods

Microorganism:

Staphylococcus aureus was isolated from soil on the basis of its ability to grow in ferulic acid containing medium. Pure cultures of these strains were maintained on a mixed medium containing both beef extract and peptone.

Medium and Culture conditions:

After growth on a mixed broth medium containing both beef extract and peptone for 5 days, 1 ml cell suspension was transferred into the 100 ml flask each containing 25 ml of minimal medium (Muheim and Lerch, 1999) along with ferulic acid as a sole carbon source. The pH of the media was adjusted to 7.2. The cultures were incubated at 35^0C and analyses were carried out on day-to-day basis upto 8 days of incubation to detect the degradation product of ferulic acid. Each experiment was carried out in triplicate.

Extraction and detection of metabolites from the culture media:

For the extraction of ferulic acid and its degradation product from the culture media, culture supernatants were prepared by centrifugation. These were acidified (pH 1-2) and extracted with equal volume of ethyl acetate. The ethyl acetate was evaporated in vacuum and residue was re-dissolved in 50% methanol. This processed culture filtrate was subjected to HPLC. Quantification of ferulic acid and its degradation products were performed at 254 nm and 310 nm. (Ghosh *et al* 2005, Sachan *et al* 2004). Compound in sample was identified by comparison with authentic standard.

Supplementation of glucose in addition to ferulic acid: In order to make high density culture of *Staphylococcus aureus***,** microorganism was allowed to grow in minimal media supplemented with glucose (0.1% w/v) as a sole carbon sources. When glucose was completely consumed by the

microorganism, ferulic acid (5.0 mM) was added into minimal medium.

3. Results and Discussion

Day basis analyses were performed to detect the product of ferulic acid bioconversion by *Staphylococcus aureus*. Vanillin was detected as the major degradation product (9.8 mg/l) after 6 days of incubation period in absence of glucose (Table 1 & Figure 1). Amount of vanillin was enhanced (45.7 mg/L) only on 48 hours of incubation period when glucose was added into the medium (Fig.2). Quantification of ferulic acid and its degradation product such as vanillin and was analyzed through HPLC. The use of additional carbon source helped in the formation of high density cultures (Oddou et al 1999) which helps in the formation of product in a shorter period of incubation period. Microorganism was pregrown on minimal media containing glucose as a carbon source. Ferulic acid utilized more rapidly when microorganism was pregrown on minimal media supplemented with glucose. Maximum accumulation of vanillin (45.7 mg/L) was observed in 48 hours of incubation period. Hence, use of the high density culture of this *Staphylococcus aureus* resulted more than four fold enhancement of vanillin formation in a shorter incubation period (48 hrs).

Table 1. Vanillin formation from ferulic acid by *Staphylococcus aureus* on a time-course basis (Without glucose).

Period of Incubation (days)	Amount of Ferulic acid (mg/l)	Amount of vanillin (mg/l)
0	520	0
1	375	0
2	165	1.2
3	18	3.4
4	11	5.7
5	5	6.9
6	1.2	8.1
7	0.65	9.8
8	0.22	5.2

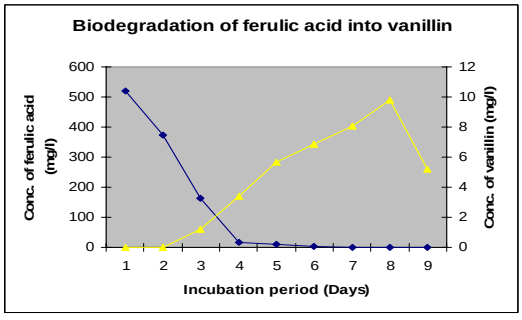

Figure 1.Time course degradation of ferulic acid and subsequent formation of vanillin by *Staphylococcus aureus* (Without glucose)

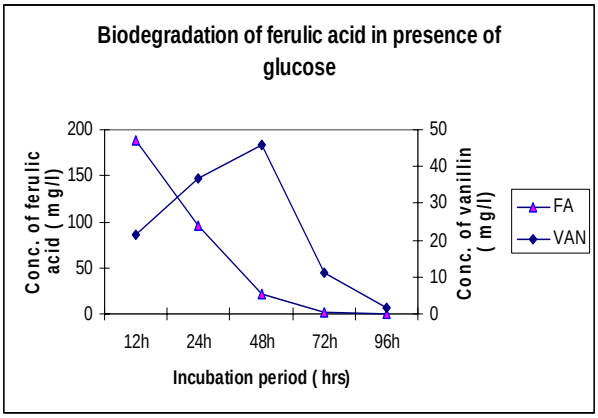

Figure 2. Time course degradation of ferulic acid and detection of vanillin in the culture media of *Staphylococcus aureus***.** (presence of glucose).

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