Standardization Of Cultural Conditions For Maximum Vanillin Production Through Ferulic Acid Degradation

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Abstract: Work has been carried out to study the standardization of different cultural conditions during the biotransformation of ferulic acid into vanillin using a Streptomyces isolate S10. Three parameters such as substrate concentration, temperature and supplementation of other carbon source (glucose) were taken into consideration. Ferulate concentrations of 5 mM, temperature of 28° C were standardized. Addition of glucose showed 5- fold increase in vanillin production. [Report and Opinion. 2009; 1(5):49-51]. (ISSN: 1553-9873).

Key words: ferulic acid, biotransformation, vanillic acid, vanillin, *p*-coumaric acid

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 (Ishii, 1997). Microbes transform hydroxycinnamic acids to their corresponding hydroxybenzoates. These benzoates are important components of natural flavours and fragrances. 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 *Streptomyces* isolate S10 to degrade ferulic acid. In this process of biotransformation, vanillin was the major degradation product. Various parameters such as substrate concentration, temperature and supplementation of other carbon source (glucose) were analyzed for maximum vanillin production.

Materials and Methods

Microorganism

Streptomyces **S10** 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 Arginine Glycerol Salt (AGS) slants **(**El-nakeeb and Lechevalier**,** 1963).

Medium and Culture conditions

After growth on AGS broth for 7 days, 1 ml cell suspension was transferred into the 100 ml flask each containing 25 ml of minimal medium (Muheim and Lerch, 1999) containing ferulic acid as a sole carbon source. The pH of the media was adjusted to 7.0. The cultures were incubated at 28^0C 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. The standard deviations of the analyses were less than 5%.

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 thin layer chromatography (TLC) and HPLC. (Ghosh *et al*. 2005; Sachan *et al*. 2004). Further confirmation was carried out using the Electron Spray Ionization Mass Spectrometric (ESI-MS).

Standardization cultural conditions

a. Concentration:

Effect of various concentrations of ferulic acid on vanillin formation was examined by flask experiments. Microorganisms were grown aerobically in minimal media containing various concentrations (1.0, 2.5, 5.0, 7.5, 10.0 mM) of ferulic acid as a sole carbon source. After 8 days of incubation, the ferulic acid utilization was carried out.

b. Temperature

Cultures were incubated at various temperatures (28 ^{o}C, 37 ^{o}C). Day basis analysis was carried out by sampling the cultures for 8 days.

c. Supplementation of other carbon source (Glucose)

In order to make high density culture of *Streptomyces* isolate S10, microorganism was allowed to grow in minimal media supplemented with glucose (0.1% w/v) as an sole carbon source. After completely consumption glucose by the microorganism, ferulic acid (5.0 mM) was added into the minimal medium.

Results and Discussion

In this case study, 5mM concentration of ferulic acid was found to be optimum for maximum vanillin production (**Fig. 1).** It was observed that a maximum amount of vanillin (10.334 mg/l) was obtained on 7th day of incubation at 28 ^{o}C **(Fig 2, Table. 1)**. In this case, microorganism consumed ferulic acid very quickly with maximum accumulation of vanillin (51.865 mg/l) after 12 h **(Fig 3, Table. 2)**. There was an increase in product (vanillin) accumulation with increase in concentration of ferulic acid up to 5mM concentration. With further increase in concentration (7.5 mM and 10 mM), there was a decrease in the product formation. The optimum temperature required for maximum vanillin production was 28 °C. A maximum amount of vanillin (10.33 mg/l) accumulated on day 7 of incubation at 28 °C in comparison to the vanillin (4.2 mg/l) production at 37 °C. Supplementation of other carbon source (glucose) at 0.1% (w/v) concentration along with ferulic acid was tested as another cultural condition for maximum amount of vanillin production. It was reported earlier that 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 consumed ferulic acid very quickly with 5 fold accumulation of vanillin (51.865 mg/l) after 12 h. In this bioconversion process in presence of glucose, vanillic acid was also detected along with vanillin. It was assumed that the amount of vanillin that accumulated in the culture medium was probably toxic for the microorganism, hence was further converted to vanillic acid in this case.

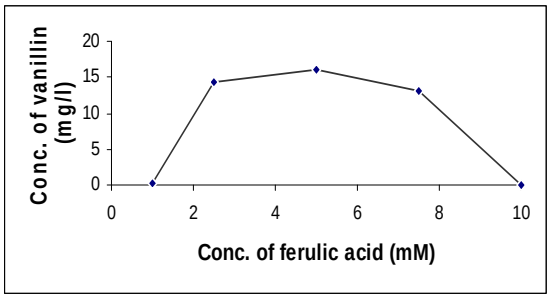

Figure 1.Time course accumulations of vanillin in the culture media of *Streptomyces* isolate **S10** at various concentrations of ferulic acid (mM).

Table 1: Time course detection of vanillin in the culture media of *Streptomyces* isolate **S10** at two different incubation temperatures.

Day	Conc. of vanillin at 28°C	Conc. of vanillin at 37°C
1st	0.00	0.00
2nd	0.483	0.109
3rd	2.481	0.264
4th	3.586	1.495
5th	3.941	3.907
6th	5.902	4.199
7th	10.334	3.814
8th	8.631	3.138

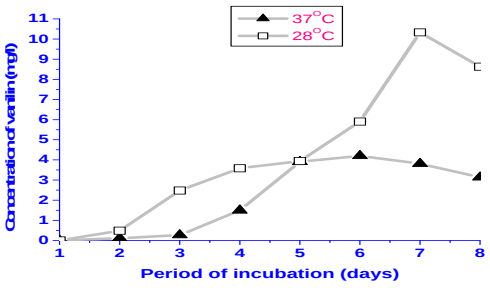

Figure 2. Time course detection of vanillin in the culture media of *Streptomyces* isolate **S10** at two different incubation temperatures.

Table 2. Time course degradation of ferulic acid (FA) and detection of vanillin (Van) and vanillic acid (VA) in the culture media of *Streptomyces* isolate **S10**.

Hours	FA (mg/l)	Van (mg/L)	VA (mg/l)
12 h	188.804	51.865	29.574
24 h	172.402	10.558	17.327
48 h	2.320	6.376	6.4
72 h	0.088	4.610	5.6

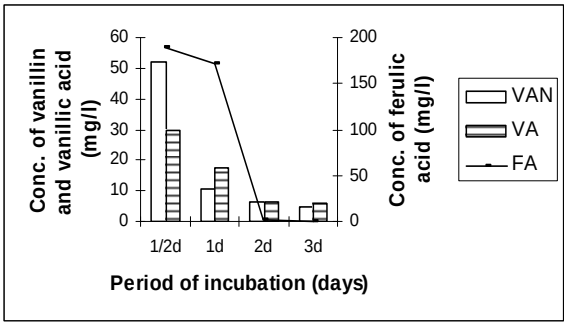

Figure 3. Time course degradation of ferulic acid and detection of vanillin and vanillic acid in the culture media of *Streptomyces* isolate **S10**.

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