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# Effect of Different Carbon Sources on Catabolism of Ferulic Acid by *Staphylococcus aureus* and Bacterial Population Analysis

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**A study was carried out on the population study of *Staphylococcus aureus* with different carbon sources during the course of ferulic acid catabolism The treatments in the study involved ferulic acid (FA), ferulic acid + glucose (FAG) and ferulic acid + biosludge (FAB) during catabolism process towards production of vanillin. Results revealed that S. aureus consumed ferulic acid rapidly with more than 4-fold increased accumulation of vanillin (45.7 mg/l) on day 2 in FAG treatment, whereas 9.8 mg/l of vanillin accumulation was found on day 7 in FA treatment. FAB treatment gave an intermediate effect on day 4 with vanillin accumulation of 22.8 mg/l. The population of bacteria in FAG medium was highest followed by FAB medium. In FA treatment, their population was lowest among all conditions. Hence, as per the production of vanillin and bacterial population, the treatments can be ranked as FAG > FAB > FA.**

**Key words***: Staphylococcus aureus,* ferulic acid, glucose, biosludge, vanillin.

Ferulic acid is a phenolic compound universally found in plant tissues and relatively abundant in their cell walls (Hartley & Harris, 1981; Harris & Hartley 1980). They are found covalently linked to polysaccharides by ester bonds and to components of lignin by ester or ether bonds (Scalbert *et al*., 1985). Ferulic acid represents up to 1.5% dry weight of the cell wall of the members of family *Poaceae*. These influence the mechanical properties like extensibility and biodegradability of the cell wall (Parker & Waldron, 1995; Jung & Deetz, 1993). Recently, the increasing interest in natural products has led to develop flavors via biotechnological processes involving microorganisms. An important attribute of microorganisms as biocatalysts in bioreactors is the ability to synthesize products in a consistent and predictable manner. Due to chemical similarity between ferulic acid and vanillin, the biotransformation of ferulic acid to vanillin was the study of interest. Vanillin is a well-known name in food processing industries mostly dairy industries, especially those of ice-cream and chocolate which imparts the flavor and aroma of vanilla. Vanillin is one of the most important aromatic and flavoring compounds used in foods, beverages, perfumes and pharmaceuticals. Considering the increasing demand for bio-products, development of flavors using microorganisms offers an alternative to their chemical ways of production (Walton *et al*., 2003).

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The current study reports the capability of *S. aureus* in bioconversion of ferulic acid to vanillin as function of its population. Effects of biosludge and glucose as carbon sources on the production of metabolites were the focus of study. The purpose of selecting glucose and biosludge is that glucose serves as a single carbon source and biosludge is a composite mixture of many carbon sources. Our hypothesis was glucose and biosludge would enhance vanillin production by *S. aureus* faster than ferulic acid alone due to extra carbon supplementation for microbial proliferation.

## MATERIALS AND METHODS

### Microorganism

*S. aureus* was isolated from soil on the basis of its ability to grow in ferulic acid containing medium. Pure cultures of these strains were obtained in a mixed nutrient medium containing both beef extract and peptone in ratio of 3:5 and incubated at room temperature. The pure cultures were maintained under refrigeration.

### Medium and culture conditions

After growth on a mixed nutrient 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 & 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 $^{o}$C and analyses were carried out on day-to-day basis up to 10 days of incubation to detect the degradation product of ferulic acid. Each experiment was carried out in triplicate.

### Separation and identification of ferulic acid and degradation products by TLC/HPLC

TLC analysis was performed as described by Dey *et al*. (2003). The plates were developed in 2% aqueous formic acid. Ferulic acid and the degradation products were viewed under a UV lamp of wavelength 310/254 nm. The bands corresponding to standards were detected on the plate. A linear isocratic solvent system was used in HPLC for the separation (Sachan *et al*., 2004). The identification of each phenolic compound was confirmed by comparing the retention times and UV-spectra with external standards.

### Substrate and treatment design

Substrate used for vanillin production was ferulic acid. Three treatments were designed as follows: (1) ferulic acid alone (FA) as the control; (2) ferulic acid + glucose (FAG) and (3) ferulic acid + biosludge (FAB).

### Ferulic acid (FA) as the control

1 ml of 5mM ferulic acid (970 mg/l) each was added to the minimal media. The media was deprived of any other carbon source.

### Ferulic acid + glucose (FAG)

Glucose (0.1% w/v) was used as the sole carbon source along with 5.0 mM ferulic acid in the media.

### Ferulic acid + biosludge (FAB)

Aerobic and activated biosludge was collected from a biological wastewater treatment plant. After collection, the biosuldge was sterilized at 121 $^{o}$C for 20 min (Guo *et al*., 2008) in order to prevent the interference by exogenous microorganisms with *S. aureus*. Biosludge of 5 % w/v was used as sole carbon source along with ferulic acid in the media.

### Enumeration of population of *S. aureus*

Population of *S. aureus* was enumerated by the help of colony counter (Bellstone digital colony counter LBH 1-LSR-246) in three different treatments. (FA, FAG and FAB).

**Table 1.** Enumeration of *S. aureus* in different treatments (FA: only ferulic acid; FAG: ferulic acid along with glucose and FAB: ferulic acid along with biosludge)

| Incubation Period ( days) | Number of viable bacteria (CFU/ml) | | |
|---|---|---|---|
| | FA treatment | FAG treatment | FAB treatment |
| 2 | 17 | 53 | 41 |
| 4 | 10 | 42 | 37 |
| 7 | 7 | 20 | 29 |
| 10 | 3 | 9 | 19 |

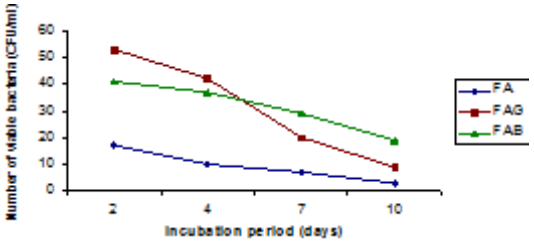


**Fig. 1.** Enumeration of *S. aureus* in different treatments (FA: only ferulic acid; FAG: ferulic acid along with glucose and FAB: ferulic acid along with biosludge)

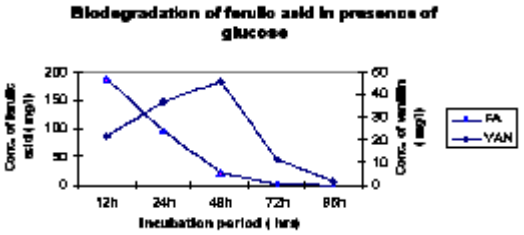


**Fig. 2.** Time course degradation of ferulic acid by *S. aureus* and detection of vanillin in presence of glucose (FAG)

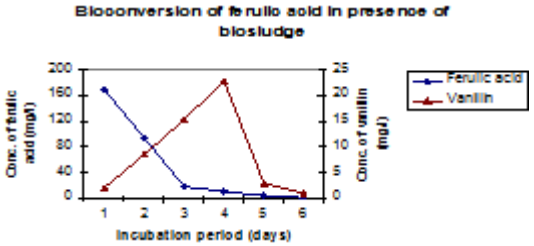


**Fig. 3.** Time course degradation of ferulic acid by *S. aureus* and detection of vanillin in presence of biosludge (FAB)

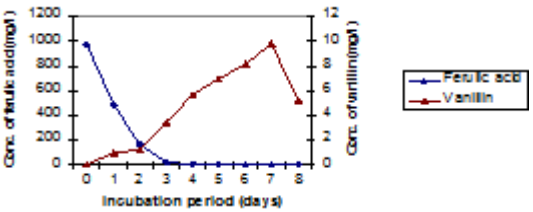


**Fig. 4.** Time course degradation of ferulic acid by *S. aureus* and subsequent formation of vanillin

## RESULTS AND DISCUSSION

*S. aureus* load was enumerated in the three treatments as represented in Figure 1. Out of these three treatments, highest staphylococcal population of was detected in FAG treatment on day 2 of incubation. This increase was due to the supplementation of glucose. Highly dense cultures of microorganism were formed in the media.

In FA treatment, population was lowest among all conditions. After day 2, population in FAG treatment was started to lower rapidly whereas a steady decrease was found in FAB. Vanillin accumulation in FAG treatment also decreased after day 2 of incubation (Fig. 2).

In FAB treatment, with almost balanced decrease in staphylococcal population, highest vanillin production was observed towards day 4 of incubation (Fig. 3).

In FA condition, degradation of ferulic acid was slow resulting maximum amount of vanillin on day 7. In this case although ferulic acid was degraded but maximum vanillin accumulation was delayed up to day 7 of incubation (Fig. 4).

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