Proof-of-Concept Engineering of *Escherichia coli* Expressing Bee Cytochrome P450 for Pesticide Detoxification

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ABSTRACT

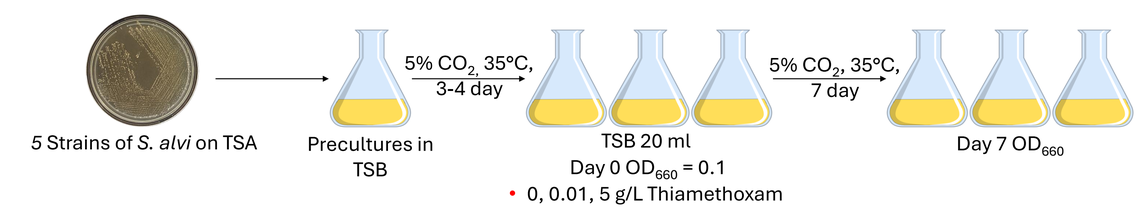
Bees play a crucial role in pollinating plants, which help increase agricultural productivity and maintain biodiversity. However, the use of pesticides in agriculture exposes bees to toxins during pollination, which may affect their health and reduce bee populations. Although bees have a natural detoxification system, it has limitations when dealing with certain pesticides, such as thiamethoxam, a widely used pesticide in Thai agriculture. This research aims to explore the potential of genetic engineering to enhance the efficacy of symbiotic microbes in bee guts to aid pesticide detoxification. The study examined the growth of *Snodgrassella alvi*, a microorganism that plays a key role in bee health by regulating the host's immune response to toxins and promoting overall bee health. First, growth yet found that 5 *S. alvi* strains, cultivated under controlled conditions with 5% CO_2 at 37ºC, exhibited different growth patterns when cultivated in the presence of thiamethoxam. The strain AD_R2A_I3, isolated from the giant honeybee (*Apis dorsata*), showed the highest growth at a concentration of 0.01 g/L thiamethoxam compared to other strains. This research further investigates the development of a genetically engineered *Escherichia coli* model for detoxifying thiamethoxam, and it showed that the expression of CYP450 burdened *E. coli* growth yet demonstrated potential in thiamethoxam detoxification. Further research on optimizing this system could contribute to developing microbial solutions for mitigating pesticide toxicity in bees.

INTRODUCTION

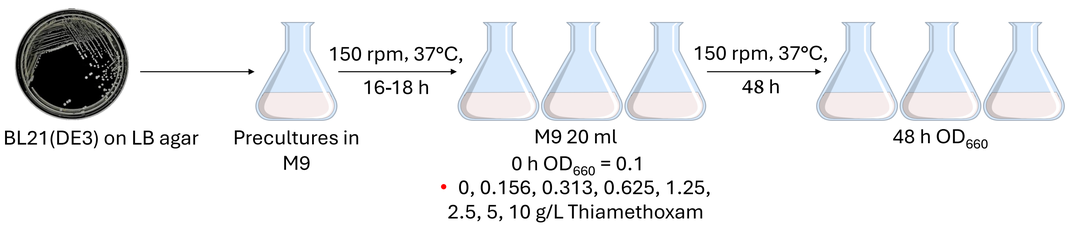
Pesticides, particularly neonicotinoids such as thiamethoxam and imidacloprid, negatively impact bee populations by contaminating pollen and nectar, disrupting the gut microbiota, and reducing beneficial bacteria (Sattayawat et al., 2024; Fairbrother et al., 2014). Bees utilize detoxification enzymes, especially cytochrome P450 monooxygenases (CYPs), to neutralize these toxins. Thiamethoxam is primarily detoxified through CYP-mediated oxidation (Wu et al., 2020). Although certain bacteria and fungi have been reported to degrade thiamethoxam, the specific CYP enzymes involved remain unidentified. This study aims to investigate CYPs for thiamethoxam detoxification particularly by engineering of *Escherichia coli* expressing bee CYP9Q1 enzyme as a proof-of-concept to enhance thiamethoxam detoxification. Additionally, 5 strains of *Snodgrassella alvi* isolated from honeybees in Thailand were selected to evaluate their potential for genetic modification and thiamethoxam degradation.

METHODS

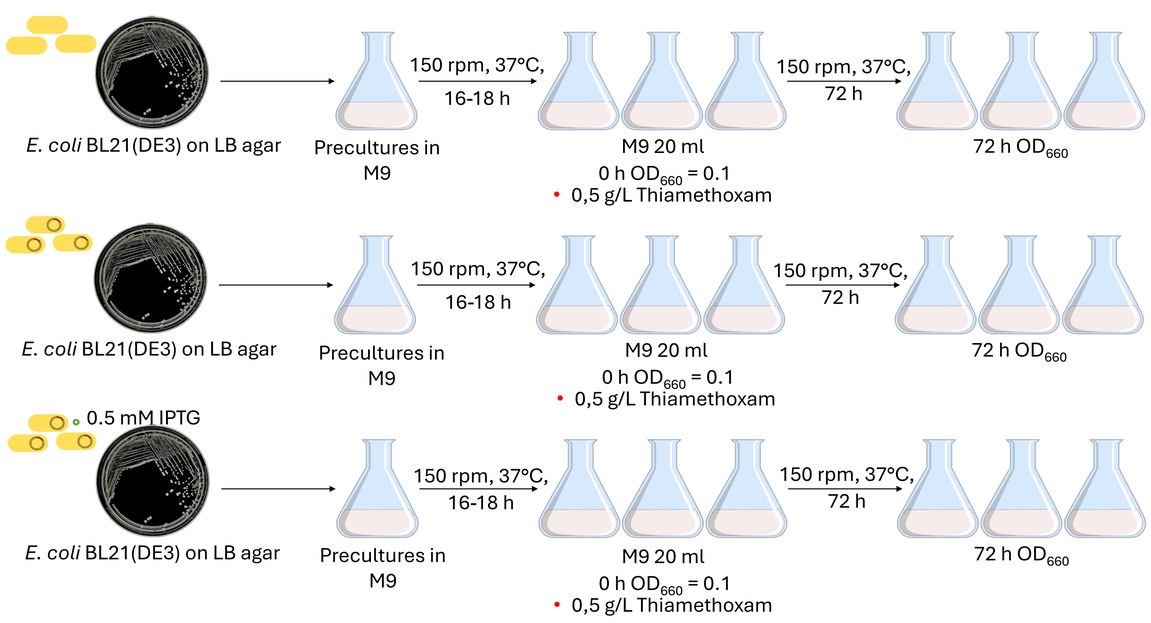
1. Toxicity of thiamethoxam on *S. alvi* in TSB liquid medium



2. Toxicity of thiamethoxam on *E. coli* BL21(DE3) in M9 liquid medium



3. Heterologous expression of CYP in *E. coli* and its thiamethoxam degradation ability



RESULTS

1. Toxicity of thiamethoxam on *S. alvi*

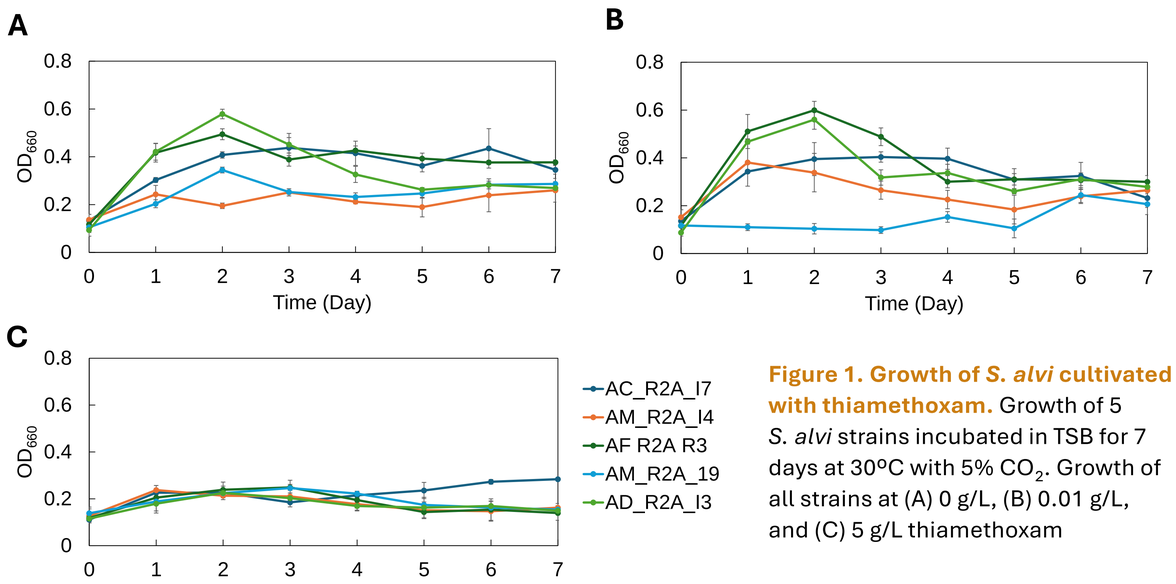


Figure 1. Growth of *S. alvi* cultivated with thiamethoxam. Growth of 5 *S. alvi* strains incubated in TSB for 7 days at 30ºC with 5% CO_2. Growth of all strains at (A) 0 g/L, (B) 0.01 g/L, and (C) 5 g/L thiamethoxam

2. Toxicity of thiamethoxam on *E. coli* BL21(DE3)

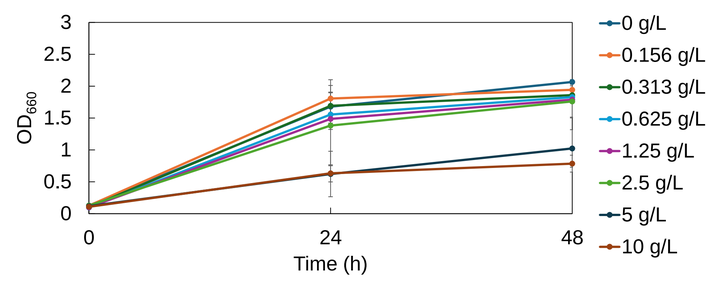


Figure 2. Growth of *E. coli* cultivated with thiamethoxam. *E. coli* BL21(DE3) was incubated for 48 h in M9 minimal medium with different concentrations of thiamethoxam

3. Heterologous expression of CYP in *E. coli* and its thiamethoxam degradation ability

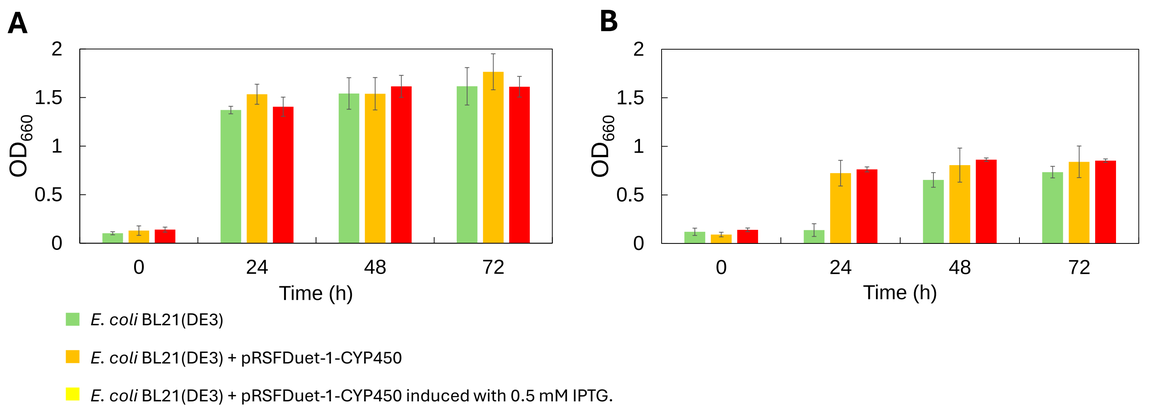


Figure 3. Effects of thiamethoxam on *E. coli* growth with and without the expression of CYP. *E. coli* BL21 (DE3) was cultivated in the resent of (A) 0 g/L Thiamethoxam and (B) 5 g/L Thiamethoxam

CONCLUSION

- **Toxicity of Thiamethoxam on *S. alvi***
  The *S. alvi* strain AD_R2A_I3, isolated from *Apis dorsata*, showed optimal growth at a Thiamethoxam concentration of 0.01 g/L. Higher concentrations inhibited its growth, suggesting the need for further investigation into the effects of Thiamethoxam on *S. alvi*.
- **Toxicity of Thiamethoxam on *E. coli* BL21(DE3)**
  At 5 g/L of thiamethoxam, *E. coli* BL21(DE3) exhibited significant growth deficiencies, indicating the harmful impact of thiamethoxam exposure.
- **Heterologous Expression of CYP in *E. coli* and Thiamethoxam Degradation**
  The study revealed that CYP450 influenced the growth of *E. coli* in both conditions; absence and presence of 5 g/L of thiamethoxam, suggesting that the expression of CYP burden cell growth. Further optimization of its expression should be investigated prior to the evaluation of its role in thiamethoxam detoxification.

REFFERENCES

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