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

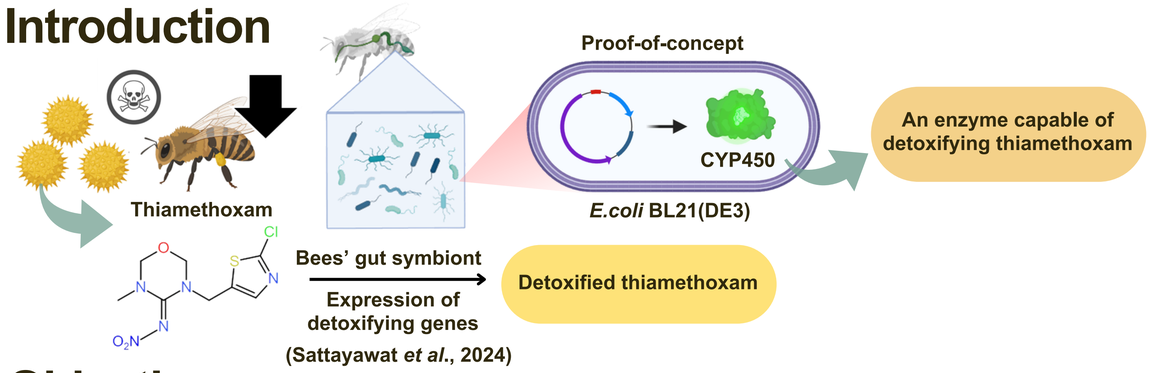
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Abstract

Bees are essential pollinators for ecosystems and agriculture; however, their populations are currently declining due to unintentional exposure to pesticides, particularly neonicotinoids such as thiamethoxam. Although bees possess endogenous detoxification enzymes, their efficiency may not always be sufficient. This study aims to develop a probiotic-based detoxification concept by genetically engineering *Escherichia coli* BL21(DE3) to express a cytochrome P450 monooxygenase (CYP450) from *Apis mellifera*. Toxicity assessment of thiamethoxam on *E. coli* BL21(DE3) revealed high tolerance, with normal growth observed at concentrations up to 2.5 g/L. However, induction of enzyme expression using IPTG proved toxic to the cells; thus, optimization tests determined 0.5 mM to be the optimal concentration. Upon enzyme expression, HPLC analysis demonstrated a significant reduction in thiamethoxam levels after 48 hours of incubation. LC-MS analysis of the dominant metabolite following detoxification identified an ion with a *m/z* of 261.0755, which corresponds to benzophenones according to the preliminary database. However, further investigation suggested that this *m/z* also corresponds to desnitro thiamethoxam ($C_8H_{12}ClN_5OS$), a derivative of thiamethoxam through nitroreduction reactions. These findings demonstrate the potential of applying bee-derived detoxifying enzymes for the development of probiotics to protect bees from pesticides in the future.

Introduction



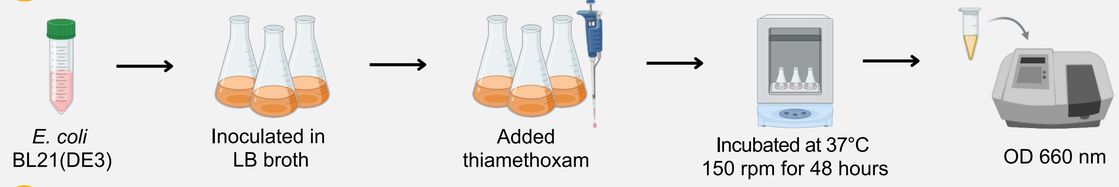
Objectives

- To optimize IPTG concentrations for CYP450 expression
- To determine the thiamethoxam depletion by CYP450-expressing *E. coli*
- To investigate the chemical profile of CYP450- expressing *E. coli* after detoxification

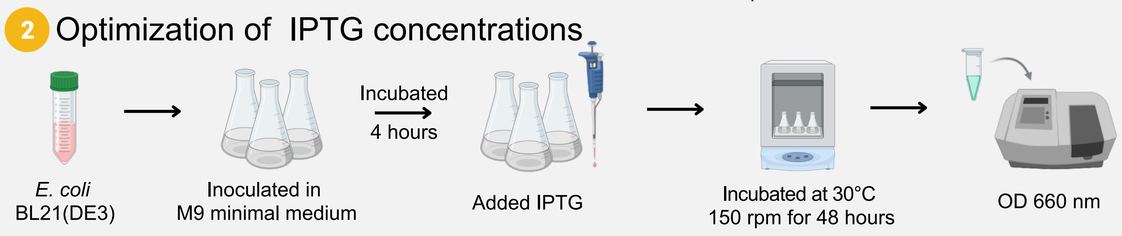
Materials & Methods

- *E.coli* BL21(DE3) carrying **pRSFDuet-1-CYP450-Kan** plasmid

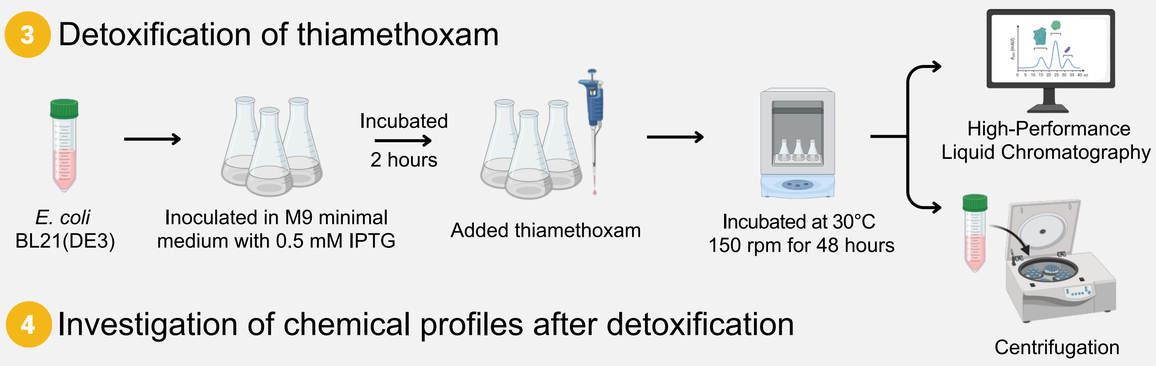
1 Investigation of the thiamethoxam toxicity on *E. coli*



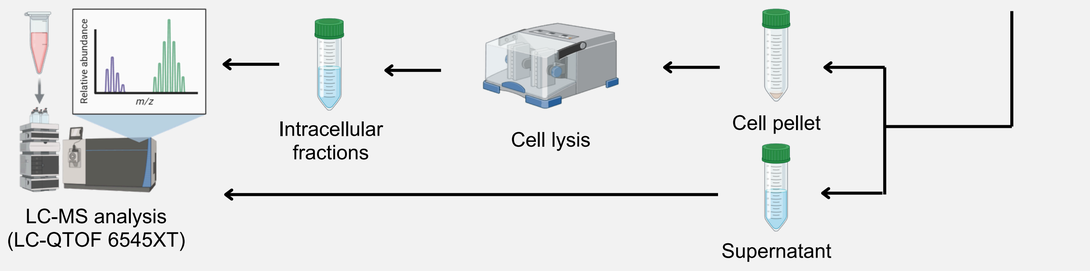
2 Optimization of IPTG concentrations



3 Detoxification of thiamethoxam



4 Investigation of chemical profiles after detoxification



Discussion and Conclusions

- Bee cytochrome P450 monooxygenase (CYP450) was succesfully expressed in *E. coli* BL21(DE3)
- The protein expression induced with 1 mM IPTG was low and inconsistent, whereas induction with 0.5 mM IPTG was optimal; however, impaired growth was observed
- HPLC analysis showed a reduction in thiamethoxam concentrations when CYP450 was expressed
- LC-MS analysis suggested that the bee CYP450 metabolizes thiamethoxam via a denitration reaction producing desnitro thiamethoxam (Boufercha *et al*., 2022)

Acknowledgements

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- SMART BEE SDGs Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

References

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- Boufercha, O., Monforte, A. R., Boudemagh, A., Ferreira, A. C., Castro, P. M. L., & Moreira, I. S. (2022). Biodegradation and Metabolic Pathway of the Neonicotinoid Insecticide Thiamethoxam by Labrys portucalensis F11. International Journal of Molecular Sciences, 23(22), 14326. https://doi.org/10.3390/ijms232214326

Results

1 Investigation of thiamethoxam toxicity on *E. coli* BL21(DE3)

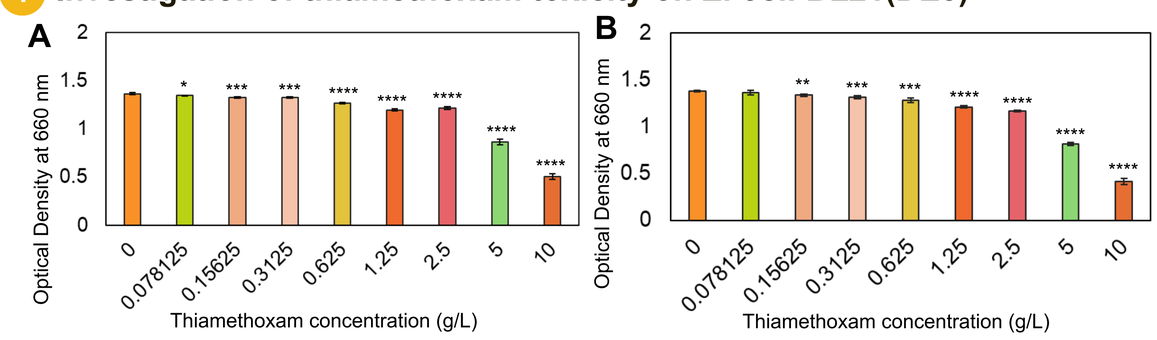


Figure 1 Toxicity of thiamethoxam on *E. coli* BL21(DE3) (A) 24 hours and (B) 48 hours. Data are mean of values from three biological replicates. Asterisks indicate statistical significance (*p-value ≤ 0.05, **p-value ≤ 0.01, ***p-value ≤ 0.005).

2 Optimization of IPTG concentrations

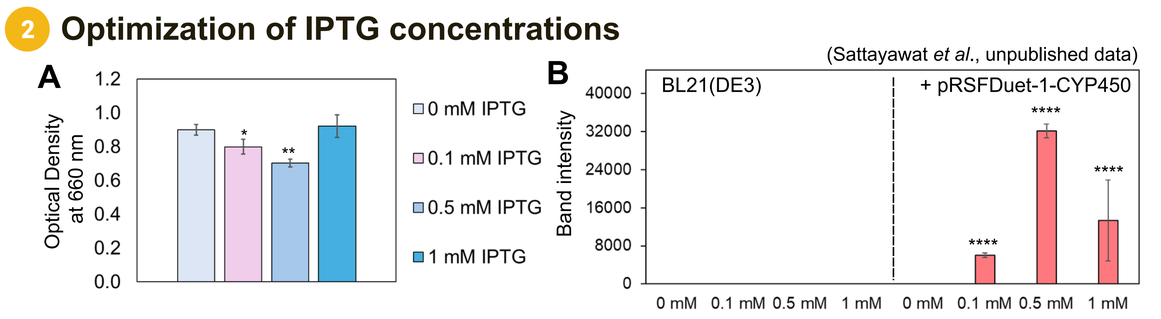


Figure 2 Heterologous expression of bee CYP450 in *E. coli* BL21(DE3) (A) The growth was observed after 4 hours of induction. (B) CYP450 band intensity. Data are mean of values from three biological replicates. Asterisks indicate statistical significance (*p-value ≤ 0.05, **p-value ≤ 0.01, ***p-value ≤ 0.005).

3 Determination of *E.coli* BL21 (DE3) growth in the presence of thiamethoxam

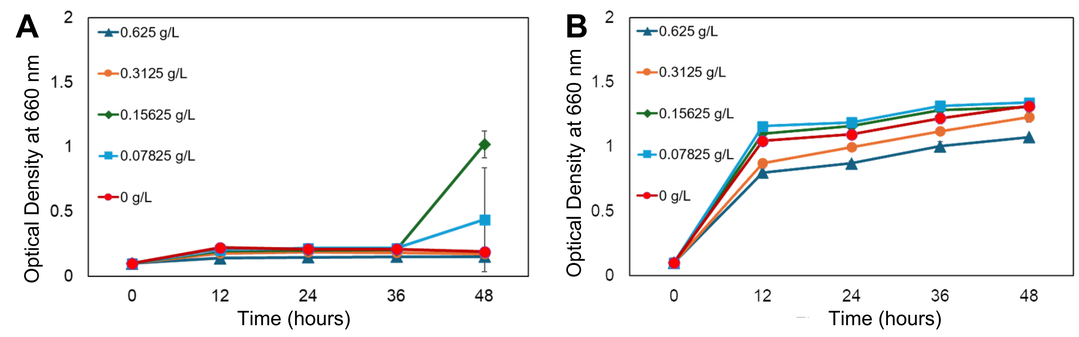
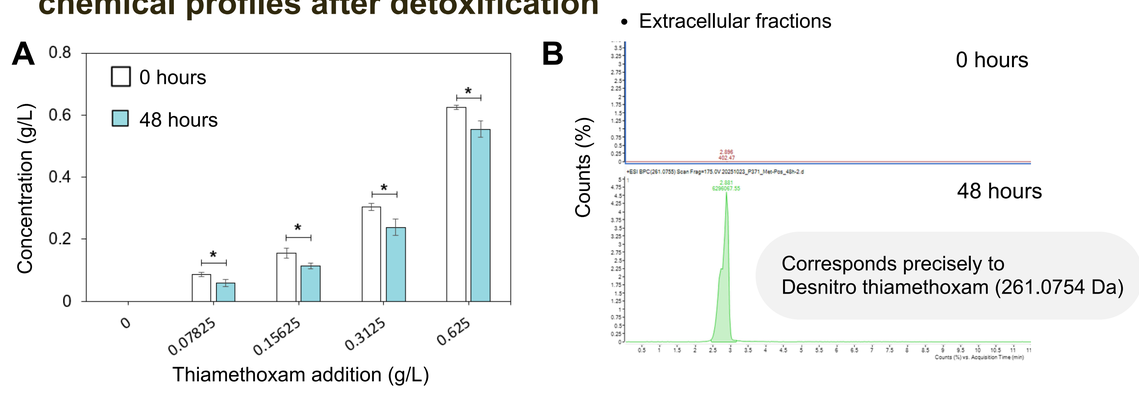


Figure 3 Growth of *E. coli* BL21(DE3) when thiamethoxam is present (A) Induced with 0.5 mM IPTG and (B) Without IPTG induction.

4 Determination of thiamethoxam depletion and investigation of chemical profiles after detoxification



C

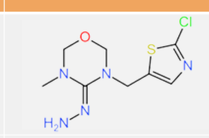
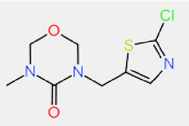
Treatment	*m/z*	RT (min)	Response	Metabolite	Structure
Supernatant (At the end of the incubation)	261.0755	3.535	6,661,137.38	low score: 2,3,4'-Trihydroxy-4-Methoxybenzophenone	
Induced sample	247.0410	3.525	285,267.60	NOA407475 (or Desnitro Thiamethoxam)	
Uninduced sample		3.535	122,216.54		

Figure 4 *E. coli* whole-cell detoxification of thiamethoxam (A) HPLC analysis of thiamethoxam from feeding assay with 0.5 mM IPTG induction. (B) LC-MS analysis of the metabolite after detoxification (C) LC-MS analysis of selected mass with the highest abundance change. Asterisks indicate statistical significance (*p-value ≤ 0.05).