Bioremediation of Two Neonicotinoid Insecticides, Thiamethoxam and Imidacloprid, by Select Bacterial Species

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Abstract

Neonicotinoid insecticides, including Thiamethoxam (THM) and Imidacloprid (IMI), have been associated with colony collapse disorder and developmental neurotoxicity. Environmental persistence of these insecticides establishes the need to develop sustainable bioremediation techniques. Phase I of this study evaluated the potential for bacterial remediation of THM and IMI by *Pseudomonas aeruginosa, Pseudomonas putida,* and *Pseudomonas fluorescens*. Bacterial utilization of these compounds as sole carbon or nitrogen sources was assessed as a function of initial THM/IMI concentration (10-90 mg/L). All three species used THM, but not IMI, as their sole carbon and nitrogen source. Based on Phase I results, Phase II was designed to characterize the kinetics of THM removal from aqueous media by *P. aeruginosa, P. putida, P. fluorescens, Alcaligenes faecalis, Escherichia coli, and Streptococcus lactis*. Residual THM and metabolites were measured using HPLC; statistical analysis was run using JMP. Results showed significant ($p<0.0001$) THM removal by *E. coli* (T_{50}=12d), *P. fluorescens* (T_{50}=18d), *P. putida* (T_{50}=19d), *P. aeruginosa* (T_{50}=23d). Removal by *A. faecalis* (T_{25}=17d) was slower, and *S. lactis* removal was minimal. THM removal showed a strong negative correlation (R^2=0.98) with an unidentified metabolite in *P. putida* and *P. fluorescens* cultures.

Introduction

FIGURE 1. 2017 Honey Nut Cheerios box. Bee removed to increase pollinator awareness.

Insect pollinators, including the honeybee, provide the means for production of over 90 commercially grown crops in North America, and are essential to 35% of food production on a global scale (Office of the Press Secretary 2014). Colony Collapse Disorder (CCD) is a contemporary phenomena characterized by mysterious vanishing of worker bee colonies. While there appears to be no singular definitive cause of CCD, a family of insecticides called the neonicotinoids (including IMI and THM) have been identified as a significant contributing factor (US EPA 2013). Neonicotinoids account for 30% of global insecticide commerce, with Imidacloprid sales surpassing $1 billion as of 2009 (Office of Policy Analysis 2015). Derived from nicotine, neonicotinoids have become increasingly popular due to their highly targeted specificity as insect neurotoxicants. While this specificity substantially reduces their toxicity to mammals, effects on off-target insects, including honeybees, has become a growing concern. Further, neonicotinoids persist in soil well beyond their agricultural usefulness, with Thiamethoxam exhibiting a half-life of ~46-300 days, depending on soil type (Gupta et al. 2008). Bioremediation, the use of microorganisms to detoxify contaminants in the environment (US EPA 2015), offers a potentially inexpensive, ecologically-friendly solution to the problem of neonicotinoid persistence. The present study assessed the potential of six bacterial species, *Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens*, *Alcaligenes faecalis, Escherichia coli,* and *Streptococcus lactis,* to bioremediate the neonicotinoids Imidacloprid and Thiamethoxam by analyzing their ability to remove these compounds from aqueous environments under laboratory conditions.

Data

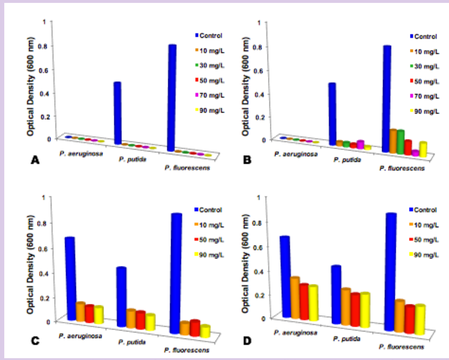

FIGURE 3. Optimization of bacterial growth conditions using Imidacloprid and Thiamethoxam as sole carbon and nitrogen sources. *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Pseudomonas fluorescens* were cultured under standard aerobic conditions at 30°C in Pseudomonas Minimal Media with (A) Imidacloprid as sole carbon source, (B) Imidacloprid as sole nitrogen source, (C) Thiamethoxam as sole carbon source, and (D) Thiamethoxam as sole nitrogen source. Bacterial growth was measured by spectrophotometry at 600 nm after 5 days for Imidacloprid and 21 days for Thiamethoxam. Control = Pseudomonas Minimal Media.

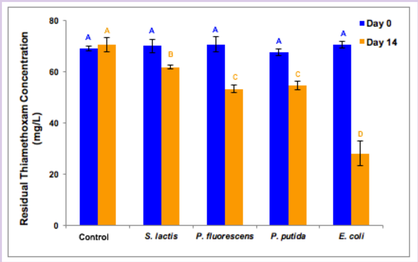

FIGURE 5. Removal of Thiamethoxam from aqueous solution by *Streptococcus lactis, Pseudomonas fluorescens, Pseudomonas putida,* and *Escherichia coli*. Bacteria were cultured under standard aerobic conditions at 30°C (*S. lactis, P. fluorescens, and P. putida*) or 37°C (*E. coli*) in half-strength nutrient broth supplemented with Thiamethoxam for 14 days. Residual Thiamethoxam levels were measured after 14 days using HPLC. Control = uninoculated media. N = 5 replicates per treatment group.

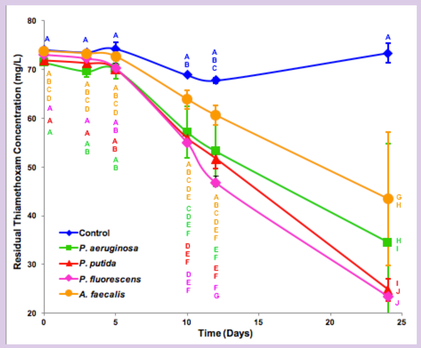

FIGURE 4. Removal of Thiamethoxam from aqueous solution by *Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens*, and *Alcaligenes faecalis*. Bacteria were cultured under standard aerobic conditions at 30°C in half-strength nutrient broth supplemented with Thiamethoxam for 24 days. Media was sampled at the indicated intervals and residual Thiamethoxam levels were measured using HPLC. Control = uninoculated media. N = 5 replicates per treatment group.

TABLE 2. Removal of Thiamethoxam from aqueous solution by selected bacterial species

Treatment	T_{25}	T_{50}	r^2 for 1st Order Fit	r^2 for 2nd Order Fit
Control	NA	NA	NA	NA
P. aeruginosa	12	23	0.99	0.97
P. putida	11	19	0.98	0.93
P. fluorescens	11	18	0.99	0.94
A. faecalis	17	NA	0.99	0.98
E. coli	8	12	NA	NA
S.lactis	NA	NA	NA	NA

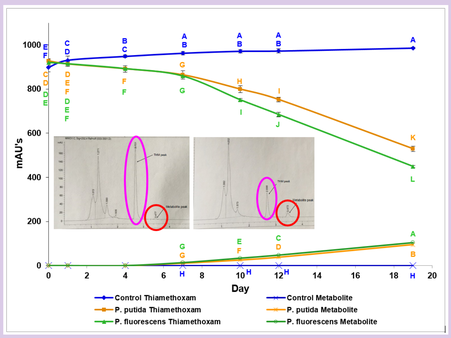

FIGURE 6. Metabolite generation from Thiamethoxam by *Pseudomonas putida* and *Pseudomonas fluorescens*. Bacteria were cultured under standard aerobic conditions at 30°C in half-strength nutrient broth supplemented with Thiamethoxam for 19 days. Media was sampled at the indicated intervals and residual Thiamethoxam and unidentified metabolite levels were measured using HPLC. Control = uninoculated media. N = 5 replicates per treatment group. Inset: HPLC chromatogram.

TABLE 3. Kinetic Parameters for Thiamethoxam Removal

Bacterial Type	Days (d)	First Order Removal Rate Constant (k_1, d^{-1})
P. fluorescens	3	0.011 ± 0.018^{IJ}
	5	0.039 ± 0.015^{GH}
	10	0.286 ± 0.046^{DE}
	12	0.448 ± 0.031^{CE}
	24	1.143 ± 0.057^{AA}
P. putida	3	0.019 ± 0.014^{IJ}
	5	0.114 ± 0.124^{FGH}
	10	0.300 ± 0.063^{DF}
	12	0.606 ± 0.377^{BD}
	24	1.066 ± 0.092^{AA}
P. aeruginosa	3	0.026 ± 0.017^{IJ}
	5	0.019 ± 0.030^{IJ}
	10	0.227 ± 0.089^{EG}
	12	0.302 ± 0.127^{EF}
	24	0.835 ± 0.478^{B}
A. faecalis	3	0.007 ± 0.006^{GH}
	5	0.015 ± 0.012^{IJ}
	10	0.144 ± 0.029^{FGH}
	12	0.197 ± 0.033^{FGH}
	24	0.560 ± 0.268^{BC}

Materials and Methods

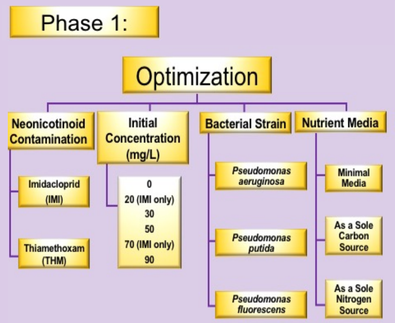

FIGURE 2: Experimental design for Phase 1

Phase 2:

TABLE 1: Experimental design for Phase 2

Bacterial Strains	*Pseudomonas aeruginosa* *Pseudomonas putida* *Pseudomonas fluorescens* *Alcaligenes faecalis* *Escherichia coli* *Streptococcus lactis*	
Neonicotinoid	Thiamethoxam (THM)	
Nutrient Media	Pseudomonas Minimal Media (PMM)	98.5 mL distilled water 1.5 mL Glycerol 0.448 g Ammonia Sulfate 0.15 g Dibasic Potassium Phosphate 0.02 g Magnesium Sulfate
	Carbon-limited PPM	98.5 mL distilled water 0.8982 g Ammonia Sulfate 0.3 g Dibasic Potassium Phosphate 0.04 g Magnesium Sulfate
	Nitrogen-limited PPM	8.5 mL distilled water 3 mL glycerol 0.3 g Dibasic Potassium Phosphate 0.04 g Magnesium Sulfate
Exposure time	Bacterial Removal Part 1	24 days
	Bacterial Removal Part 2	14 days
	Metabolite Study	19 days
Temperature	30°C	
Sampling Interval	Bacterial Removal Part 1	0, 3, 5, 10, 12, 24 days
	Bacterial Removal Part 2	0, 14 days
	Metabolite Study	0, 1, 4, 7, 10, 12, 19 days
Analyses	Bacterial growth	Thermo Scientific Genesys 20 by measuring optical density at 600nm
	HPLC – Analysis	Aligent 1260 Infinity HPLC was used for analysis with a Supercoil LC-18DB 15 cm 4.6 mm (ID), 5 µm particle size column as a stationary phase Mobile phase consisted of 70% deionized water and 30% acetonitrile Diode array detector at 252 nm
	HPLC – THM	Standard curve concentration from 0–160 mg/L with THM
	HPLC – Metabolites	Standard curves created 0-160 mg/L with clothianidin, N-desmethylthiamethoxam and nitroguanine.
Statistics	All statistical tests were carried out using JMP Pro13. Two way ANOVA followed by mean comparison was carried out using the Tukey Kramar Honest Significant Difference (HSD) test.	
Kinetics	Residual Thiamethoxam data is fit to both first order and second order kinetic models and rate constants were calculated as function of bacterial type and compared at 95% confidence interval.	

Results

Figure 1:

- IMI showed less growth than THM when used as the sole carbon and sole nitrogen source
- All organisms grew better using THM as their sole nitrogen source than as their sole carbon source
- *P. putida* showed the greatest amount of growth among experimental groups compared to the control groups for the THM treatment

Figure 2:

- *P. fluorescens, P. putida, P. aeruginosa and A. faecalis* exhibited decreases in THM concentrations by 67%, 65%, 52% and 39% respectively on day 24

Figure 3:

- *E. coli* showed a 60% reduction in residual THM concentration over 14 days
- *S. lactis* showed a 12% reduction in residual THM concentration over 14 days

Figure 4:

- As THM concentration decreased the metabolite concentration increased with both *P. fluorescens* and *P. putida*

Discussion

Experimental bacteria samples exhibited greater growth using THM compared to IMI, resulting in the continuation of THM treatment into phase II testing. The *Pseudomonas* and *Alcaligenes* species assessed all exhibited a significant decrease in residual THM after 24 days. In order to determine a negative control, *S. lactis* and *E. coli* were also assessed for their ability to remove THM. Interestingly, *E. coli* exhibited the highest level of THM removal of all bacteria tested. *S. lactis* exhibited minimal, albeit statistically significant, removal. The metabolite study showed that as the THM concentration decreased, the concentration of unidentified metabolite increased, suggesting metabolism of THM by the bacteria. Overall, this shows that select bacteria are able to degrade THM, indicating a potential bioremediation method for the removal of persistent neonicotinoids that contribute to Colony Collapse Disorder.

References

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Image Source: Honey Nut Cheerios Bee Goes Missing [Internet]. Consumerist; c2017 [updated 2017 March 9; cited 2017 April 11]. Available from: https://consumerist.com/2017/03/09/honey-nut-cheerios-bee-goes-missing-to-highlight-vanishing-bee-colonies/

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Acknowledgements

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