Macrothink Institute™

Journal of Biology and Life Science
ISSN 2157-6076
2019, Vol. 10, No. 1

Whole Genome Sequence of *Sphingobacterium* sp. G1-14, a Strain With Effective Paichongding Biodegradation

Peng Yin (Corresponding author)
Laboratory of Applied Microbiology, School of Pharmaceutical Engineering & Life Science, Changzhou University, Changzhou, China
Email: yinpengdevil@foxmail.com
Suyu Liu
Laboratory of Applied Microbiology, School of Pharmaceutical Engineering & Life Science, Changzhou University, Changzhou, China
Email: 490385680@qq.com
Xingke Wu
Laboratory of Applied Microbiology, School of Pharmaceutical Engineering & Life Science, Changzhou University, Changzhou, China

Email: 1092678638@qq.com

Received: December 6, 2018 Accepted: December 24, 2018

doi:10.5296/jbls.v10i1.14000 URL: https://doi.org/10.5296/jbls.v10i1.14000

Abstract

In this study, we obtained a Paichongding (RR/SS-IPP) degrading *Sphingobacterium* sp. G1-14 by UV irradiation of the original strain G1-13. This new mutant strain showed excellent RR/SS-IPP degradation performance, and the degradation of ratio was up to 30 per cent after 7 days. Subsequently, we determined the mutant strain G 1-14 as *Sphingobacterium* based on the phylogenomic analyses. The circular chromosome of *Sphingobacterium* sp. G1-14 was presented in this paper by Illumina Hiseq platform combined with a third-generation sequencing platform. 5583 protein-coding sequences of the complete genome sequence were obtained, which is beneficial to deduced genes related to RR/SS-IPP degradation.

Keywords: Paichongding, *Sphingobacterium*, Biodegradation, Biodegradation pathway, Whole genome

1. Introduction

Insecticide plays an increasingly important role in modern agriculture for its highly resistance to farmland pests. In the past two or three decades, neonicotinoid insecticides have become one of the largest sales insecticides in more than 120 countries around the world. As a representative of insecticide, Paichongding (IPP, 1-((6-chloropydidin-3-yl)methyl)-7-methyl-8-nitro-5propoxy-1,2,3,5,6,7-hexahydroimidazo[1,2-a-]-pyridine), was developed by Jiangsu Kwin Co., Ltd. and East China University of Science and Technology in 2008 and showed excellent activity for anti-imidacloprid insects(Cai et al., 2015; Qin et al., 2012; H. Y. Wang et al., 2013). IPP is considered as the succedaneum of imidacloprid which has negative influence on wild bumble bee populations (Fu, Zhang, et al., 2013; Whitehorn, O'Connor, Wackers, & Goulson, 2012). In China, 10% IPP suspensions have been sprayed in farmland for pest prevention such as alfalfa aphids and brown planthopper since 2009(Fu, Wang, et al., 2013). IPP is expected to reach 1,000 tons in few years, which may pose a significant threat to soil and water (Cai et al., 2015). Many studies have demonstrated that microorganisms play vital roles in pesticide degradation (MAE, MARIT, AUSMES, KOIV, & HEINAR, 1993; Vaitekunas, Gasparaviciute, Rutkiene, Tauraite, & Meskys, 2016). Remarkably, Cai et al. has reported that the IPP could be biodegraded by *Sphingobacterium* sp. P1-3(Cai et al., 2015).

At present, genes and enzymes related to the degradation of RR/SS-IPP have not been reported. Based on the newly selected RR/SS-IPP efficient degrading bacteria, this study provides insights into the degradation mechanism of RR/SS-IPP from a genetic perspective through the whole-genome sequencing and bioinformatics analysis combined with liquid chromatography tandem high-resolution mass spectrometry (LC-MS/MS).

2. Material and Methods

2.1 Chemicals and Microorganism Strain

Paichongding, (IPP, chemical purity 98.3%; Formula Weight, FW 366. Fig. 1) was obtained from Jiangsu Kesheng Company Ltd., HPLC grade methanol and acetonitrile were purchased from Burdick & Jackson (MI, USA). All other reagents and common chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Company (Shanghai, China).

Sphingobacterium sp. G1-14 (GenBank accession numbers: KP657689, CGMCC No. 10454) was irradiated from the original strain G1-13, which was isolated from soils in the south of Changzhou, Jiangsu province, China. It had good IPP-degrading activity and could use IPP as a sole carbon and energy source. *Sphingobacterium* sp. G1-14 was cultivated aerobically at 30 ^{o}C on Luria-Bertani (LB) broth. Stock cultures were stored in 20% glycerol at -80 ^{o}C.

2.2 Biodegradation Assays

To illustrate the biodegradation pathway of IPP in aquatic system, 250 mL glass flasks were used in all the degradation study and 150 l of IPP stock solution (20 g l^{-1}) was added into 100 ml of BMM solution to give a final concentration of 30 mg l^{-1}. And all the tests are three

replicates. After culture for 72 h, cells of *Sphingobacterium* sp. G1-14 were harvested by centrifugation (21,000×*g*, 4 °C, 10min) and washed twice with 0.85% (w/w) sodium chloride solution.

2.3 Extraction and Pretreatment

The cultivation was collected by centrifugation (21,000×*g*, 4 °C, 10min). The supernatant was extracted with dichloromethane three times. The recovery of IPP ranged from 91.22% to 107.29, the relative standard deviation (RSD) was less than 7.58%. The results showed that the extraction method is feasible and satisfactory for the analysis of IPP residues. The dichloromethane extracts were combined and concentrated through vacuum rotary evaporator at 40 °C to almost dry and then the extracts were dissolved in 1 ml of methanol. IPP and its biodegradation intermediates were monitored and analyzed.

2.4 Genomic DNA Extract and Sequence

The commercial kits (PureLink™ Microbiome DNA Purification Kit, ThermoFisher Scientific) were used for extracting and purifying of genomic DNA from *Sphingobacterium* sp. G1-14. The genomic DNA was sequenced using Illumina Hiseq 2500 at BGI company (Shenzhen, China).

3. Results

As a chiral insecticide, IPP can be divided into four stereoisomers according to two asymmetrically substituted C atoms (**Fig. 1**). Compared with enantiomers RS-IPP and SR-IPP, enantiomers RR-IPP and SS-IPP exhibit higher stablility and difficulty in degradation (Fu, Wang, et al., 2013; J. Wang et al., 2016). In this study, we found a gram-negative mutant strain G1-14 from G1-13 through UV mutagenesis. As is shown in **Fig 2**, G1-14 in culture plates has higher growth rate and larger single colonies than G1-13. After seven days of fermentation, the IPP degradation rate was about 30%, which was superior to original strain significantly. The phylogenetic tree based on 16S rRNA gene sequence was constructed using MEGA (v5.1) (**Fig. 3**). It could reveal the evolutionary status of *Sphingobacterium* sp. G1-14.

Journal of Biology and Life Science
ISSN 2157-6076
2019, Vol. 10, No. 1

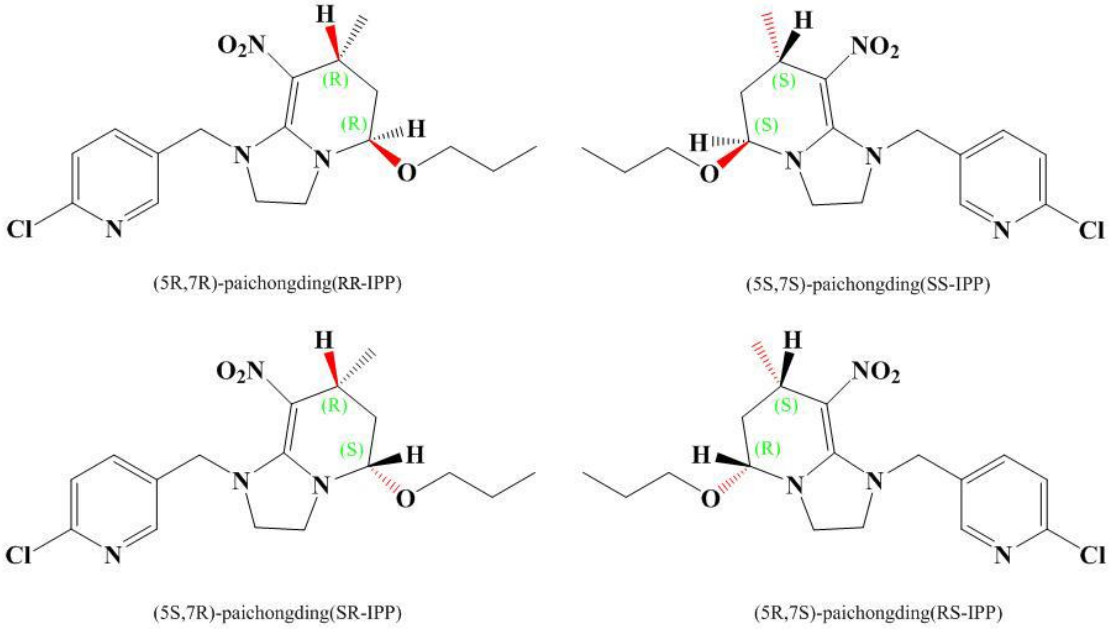

Figure 1. Four stereoisomers structures of Paichongding (IPP): (A) 5R,7R-IPP (RR-IPP), (B) 5S,7S-IPP (SS-IPP), (C) 5S,7R-IPP (SR-IPP), and (D) 5R,7S-IPP (RS-IPP) and the chiral centers have been marked R or S

Figure 2. Basal medium plates colonies of strains G1-13 / G1-14 and the IPP degradation ratio by strains G1-13 / G1-14 within seven days

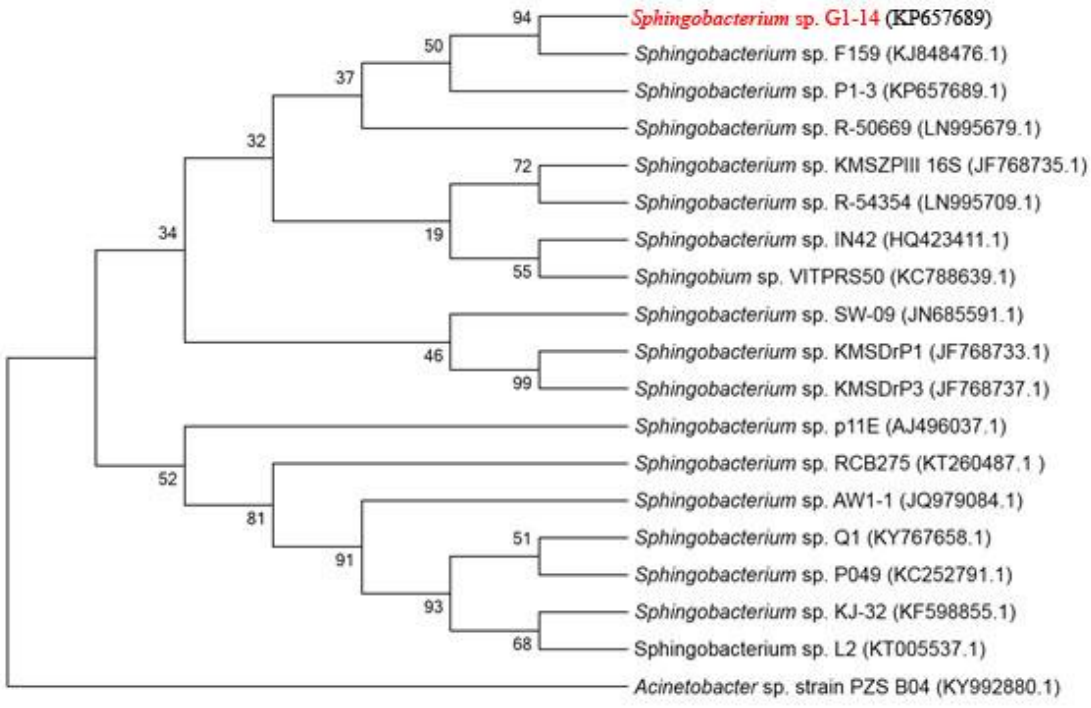

Figure 3. The phylogenetic tree was constructed based on 16S rRNA gene sequences showing the relationship between *Sphingobacterium* sp. G1-14 and other *Sphingobacterium* species. The neighbor-joining method would help to inferred the evolutionary history of *Sphingobacterium* sp. G1-14

Our group attempted to speculate the conceivable metabolic pathway of RR/SS-IPP by *Sphingobacterium* sp. G1-14 though the liquid chromatography tandem high-resolution mass spectrometry (LC-MS/MS). Owing to the complicated mechanism of RR/SS-IPP, the whole genome sequence was added to provide insights about the degradation mechanism of RR/SS-IPP from the genetic point of view. The *Sphingobacterium* sp. G1-14 was incubated at 30 ℃, 180 revolutions min^{-1} in 100 mL of LB medium. The genomic DNA was extracted using the Takara DNA kit and DNA concentration was measured using Qubit3.0 to ensure DNA quality for the subsequent experiments. Illumina HiSeq 2500 system combined with Pacfic Biosciences SMRT (PacBio RSII) have been used to complete genome sequence. The Illumina PE library and PacBio library generated 2021 Mb data (14,532,302 reads with 335 bp average coverage) and 625 Mb data (83839 subreads with 7,453 bp average length) respectively. We assembled the genome into one contig based on the reads obtained from Illumina PE library and PacBio library by SOAPdenovo (v2.04) and Celera Assembler (v8.0). GapCloser (v1.12) was used to complete the gap closure and confirm a 6,325,678 bp chromosome with circular topology(Koren et al., 2012) . The function annotations of protein-coding genes (predicted by Glimmer 3.0) were obtained from BLASTP with COG, KEGG and Swiss-Prot databases (Delcher, Bratke, Powers, & Salzberg, 2007). Finally, we indentified the rRNA and tRNA contained in the genome using RNAmmer (v1.2) and tRNAscan-SE (v1.3.1). The assembly result of *Sphingobacterium* sp. G1-14 genome revealed

that the circular chromosome contained 6,325,678 bp with G+C content of 39.83%. 5583 CDSs, 21 rRNA and 85 tRNA were identified in the chromosome respectively. The summary genome features of *Sphingobacterium* sp. G1-14 were summarized in **Table 1** and **Fig 4**.

Table 1. *Sphingobacterium* sp. G1-14 genome features

Features	G1-14
Genome size (bp)	6,325,678
GC content (%)	39.83
Numbers of CDSs	5583
Numbers of rRNAs	21
Numbers of tRNAs	85
GeneBank accession	CP021381

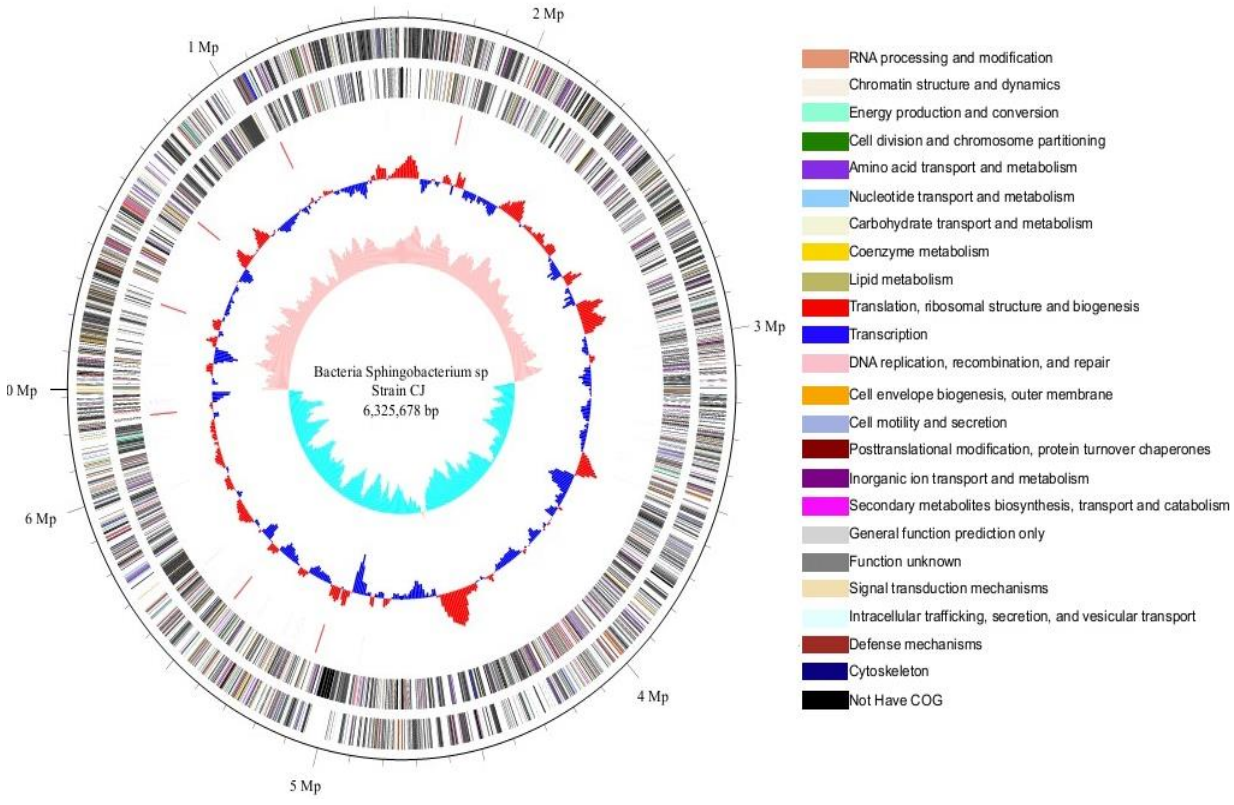

Figure 4. Genomic circle map of *Sphingobacterium* sp. G1-14. From the outside circle to the center, circle (1) shows the genomic size and each scale is 0.5Mb. Circle (2) and circle (3) represent the predicted CDSs on negative for the positive chain and negative chain, colors according to COG functional categories. Circle (4) indicates the rRNA and tRNA genes. Circle (5) shows the GC content

4. Discussion

In combination with deduced metabolic pathways (J. Wang et al., 2016), we found that: (i) IPP and imidacloprid are structurally consistent in Phase I (**Fig 5**). KEGG pathway analysis suggested that CYP450 enzymes were not exist in the genome sequence. CYP450s were the only proven enzymes that involved in imidazole ring hydroxylation in imidacloprid with stable chloropyridine ring (Casida, 2011; Fu, Zhang, et al., 2013; Schulz-Jander, Leimkuehler, & Casida, 2002) . Our results deduced that the hydroxylation of IPP occurs in the tetrahydropyridine ring based on six hydroxylases in the genome. (ii) According to the KEGG database, MqnB was found in the chromosome of G1-14 which could hydrolyze the C-N bond attached to the imidazole (Hiratsuka et al., 2008). The formation of I1 and I2 were obtained from the removal of C-N bonds between 2-chloro-5-ethylpyridine and 8-amino octahydroimidazo [1,2-alpha] -pyridin-7-ol. Based on these observations,we hypothesized that the break of this C-N bond was mainly due to the candidate enzyme MqnB.

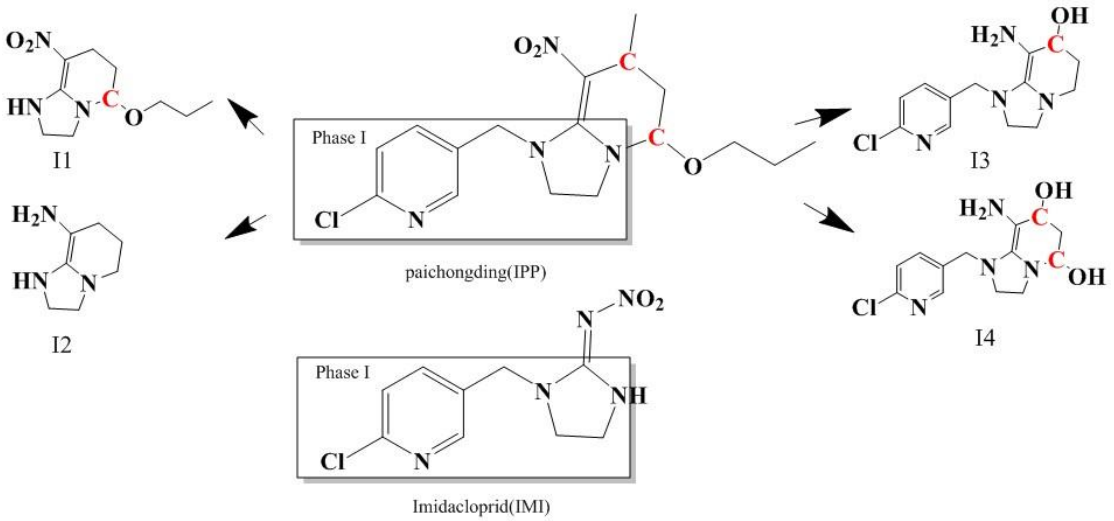

Figure 5. Paichongding (IPP) and Imidacloprid (IMI) Chemical Structural Formulas and RR/SS-IPP metabolites (I1, I2, I3 and I4) by *Sphingobacterium* sp. G1-14.

5. Conclusion

In this study, we obtained the whole-genome sequence of the *Sphingobacterium* sp. G1-14. Through bioinformatics analysis, hydroxylation of RR/SS-IPP may occur on relatively active tetrahydropyridine rings. From the metabolic point of view, the candidate enzyme MqnB might be responsible for the removal of the chloropyridine ring.

Competing interests

The authors declare that they have no competing interests.

Funding

The research was financially funded by grants from the National Natural Science Foundation of China (NSFC) (Project 11275033) and Natural Science Foundation of Jiangsu Province (BK20151185).

Authors' contributions

PY, SL, WX and JG carried out the experiments; PY and XZ wrote the paper; XZ and ZC designed the research; ZC obtained the funding. All authors read and approved the final manuscript.

References

Cai, Z. Q., Zhang, W. J., Li, S. S., Ma, J. T., Wang, J., & Zhao, X. Y. (2015). Microbial Degradation Mechanism and Pathway of the Novel Insecticide Paichongding by a Newly Isolated Sphingobacterium sp P1-3 from Soil. *Journal of Agricultural and Food Chemistry, 63*(15), 3823-3829. https://doi.org/10.1021/acs.jafc.5b00706

Casida, J. E. (2011). Neonicotinoid Metabolism: Compounds, Substituents, Pathways, Enzymes, Organisms, and Relevance. *Journal of Agricultural and Food Chemistry, 59*(7), 2923-2931. doi:10.1021/jf102438c

Delcher, A. L., Bratke, K. A., Powers, E. C., & Salzberg, S. L. (2007). Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics, 23*(6), 673-679. https://doi.org/10.1093/bioinformatics/btm009

Fu, Q. G., Wang, Y. C., Zhang, J. B., Zhang, H. X., Bai, C., Li, J. Y., ... Li, Z. (2013). Soil Microbial Effects on the Stereoselective Mineralization, Extractable Residue, Bound Residue, and Metabolism of a Novel Chiral Cis Neonicotinoid, Paichongding. *Journal of Agricultural and Food Chemistry, 61*(32), 7689-7695. https://doi.org/10.1021/jf4015153

Fu, Q. G., Zhang, J. B., Xu, X. Y., Wang, H. Y., Wang, W., Ye, Q. F., & Li, Z. (2013). Diastereoselective Metabolism of a Novel Cis-Nitromethylene Neonicotinoid Paichongding in Aerobic Soils. *Environmental Science & Technology, 47*(18), 10389-10396. https://doi.org/10.1021/es4023738

Hiratsuka, T., Furihata, K., Ishikawa, J., Yamashita, H., Itoh, N., Seto, H., & Dairi, T. (2008). An alternative menaquinone biosynthetic pathway operating in microorganisms. *Science, 321*(5896), 1670-1673. https://doi.org/10.1126/science.1160446

Koren, S., Schatz, M. C., Walenz, B. P., Martin, J., Howard, J. T., Ganapathy, G., . . . Phillippy, A. M. (2012). Hybrid error correction and de novo assembly of single-molecule sequencing reads. *Nature Biotechnology, 30*(7), 692-+. https://doi.org/10.1038/nbt.2280

MAE, A. A., MARIT, R. O., AUSMES, N. R., KOIV, V. M., & HEINAR, A. L. (1993). Characterization of a new 2,4-dichlorophenoxyacetic acid degrading plasmid pEST4011: physical map and localization of catabolic genes. *Journal of General Microbiology*(139), 3 165-163 170.

Qin, X. W., Chen, Y., Zhang, J., Liu, J., Liu, Q., Yuan, F. H., & Zhang, R. J. (2012). Toxic Effects of Paichongding on Brown Planthopper, Nilaparvata lugens (Stal) (Homoptera: Delphacidae). *Journal of Entomological Science, 47*(4), 297-308.

Schulz-Jander, D. A., Leimkuehler, W. M., & Casida, J. E. (2002). Neonicotinoid insecticides:

Reduction and cleavage of imidacloprid nitroimine substituent by liver microsomal and cytosolic enzymes. *Chemical Research in Toxicology, 15*(9), 1158-1165. https://doi.org/10.1021/tx0200360

Vaitekunas, J., Gasparaviciute, R., Rutkiene, R., Tauraite, D., & Meskys, R. (2016). A 2-Hydroxypyridine Catabolism Pathway in Rhodococcus rhodochrous Strain PY11. *Appl Environ Microbiol, 82*(4), 1264-1273. https://doi.org/10.1128/AEM.02975-15

Wang, H. Y., Yang, Z., Liu, R. Y., Fu, Q. G., Zhang, S. F., Cai, Z. Q., . . . Li, Z. (2013). Stereoselective uptake and distribution of the chiral neonicotinoid insecticide, Paichongding, in Chinese pak choi (Brassica campestris ssp chinenesis). *Journal of Hazardous Materials, 262*, 862-869. https://doi.org/10.1016/j.jhazmat.2013.09.054

Wang, J., Chen, J., Zhu, W. J., Ma, J. T., Rong, Y., & Cai, Z. G. (2016). Isolation of the Novel Chiral Insecticide Paichongding (IPP) Degrading Strains and Biodegradation Pathways of RR/SS-IPP and SR/RS-IPP in an Aqueous System. *Journal of Agricultural and Food Chemistry, 64*(40), 7431-7437. https://doi.org/10.1021/acs.jafc.6b02862

Whitehorn, P. R., O'Connor, S., Wackers, F. L., & Goulson, D. (2012). Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production. *Science, 336*(6079), 351-352. https://doi.org/10.1126/science.1215025