**Deciphering Alkaloids Bioconversion to High-Added Value Chemicals: the Metabolic Route of Lupanine Degradation in *Pseudomonas Putida* LPK411.**

Stella Parmaki1, Argyro Tsipa1, Ioannis Vyrides1, Ana Mota2, Raquel A. M. Teixeira3, Frederico C. Ferreira2, Carlos A.M. Afonso3, Michalis Koutinas1,\*

*1 Department of Environmental Science & Technology, Cyprus University of Technology, 30 Archbishop Kyprianou Str., 3036, Limassol, Cyprus; 2 Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Tecnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001, Lisbon, Portugal; 3 Research Institute for Medicines (iMed. ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal*

*\*Corresponding author: michail.koutinas@cut.ac.cy*

**Highlights**

* Systematic biodegradation kinetics of lupanine enantiomers in *P. putida* LPK411.
* A different metabolic pathway for each enantiomer exists.
* Systematic transcriptional kinetics of gene expressing lupanine hydroxylase.
* Systematic transcriptional kinetics of global genes in lupanine biodegradation.

**1. Introduction**

Lupanine constitutes the main quinolizidine alkaloid contained in *Lupinus albus* seeds. Owing to the useful functionalities of its asymmetric structure, this toxic compound has attracted considerable attention in biotechnological industries and may serve as a starting material for semi-synthesis of a range of novel high added-value compounds [1]. *L. albus* snack manufacturing generates excess of wastewater containing lupanine, while the most common process for lupanine elimination requires exhaustive boiling and leaching of seeds in water [2]. Biodegradation is a promising, greener alternative for detoxification of the wastewater generated. Thus, the objective of this study was to decipher the metabolism of lupanine biodegradation in *Pseudomonas putida* LPK411.

**2. Methods**

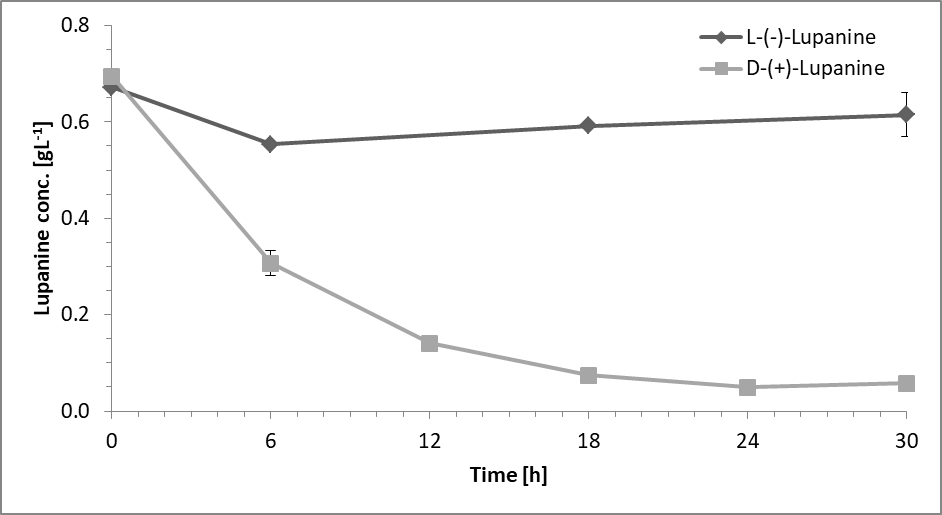
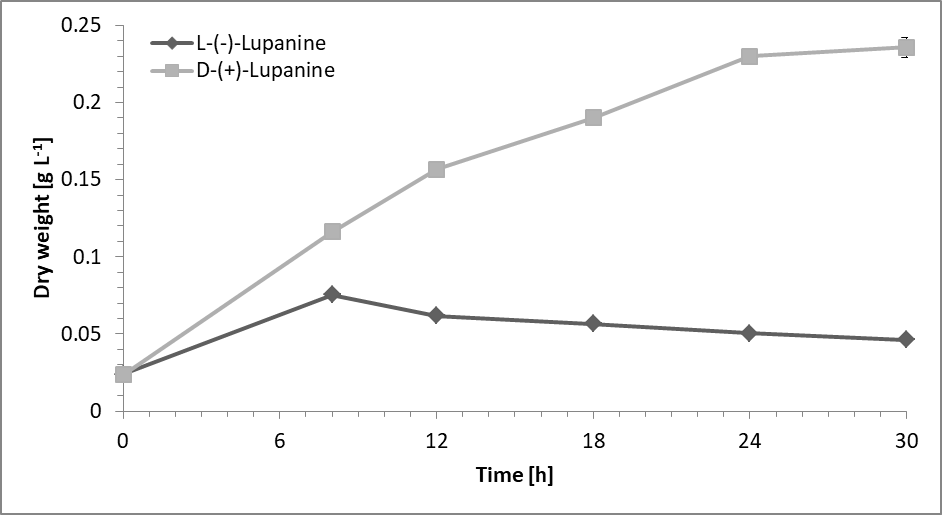
Microbial cultures: Lupanine enantiomers’ biodegradation was investigated during fermentation of *P. putida* LPK411 using initial D-(+)-lupanine and L-(-)-lupanine concentration of 0.75 g L-1 respectively. The cultures were maintained at 31 oC, pH 7 and 100 rpm.

Analytical techniques: Culture samples were withdrawn at regular intervals and biomass concentration was determined by absorbance at 600 nm on a UV/VIS spectrophotometer. Lupanine concentration was measured using GC analysis [3].

Molecular biology techniques: Total RNA isolation, cDNA synthesis and Quantitative Real-Time Polymerase Chain Reaction (Q-RT-PCR) was conducted as previously described [4].

**3. Results and discussion**

Lupanine in nature exists in a racemic mixture of two enantiomers (D-(+)-lupanine and L-(-)-lupanine) [5]. Biodegradation kinetics of D-(+)-lupanine and L-(-)-lupanine was investigated during fermentation of *P. putida* LPK411. Two batch experiments were performed. The results demonstrated that *P. putida* LPK411 can degrade D-(+)-lupanine, while L-(-)-lupanine concentration was only slightly reduced (Fig. 1). The latter indicates a different metabolic pathway for each enantiomer [6]. Moreover, LPK411 fermentations fed with racemic lupanine clarified that the strain is capable of achieving more than 95% enantiomeric excess of L-(-)-lupanine enabling the fermentative production of enantiopure L-(-)-lupanine, which avoids the application of other costly and laborious chemical methods.



**Figure 1.** Lupanine enantiomers biodegradation by *P. putida* LPK411. (A) Microbial growth (expressed as dry cell weight), (B) concentration of D-(+)-lupanine and L-(-)-lupanine during fermentation.

Thus far, only a few studies have focused on metabolic mechanisms of quinolizidine alkaloids, such as lupanine. Lupanine hydroxylase catalyses the first reaction of lupanine biodegradation in *P. putida*. The specific enzyme is active only upon D-(+)-lupanine [6] biodegradation. Moreover, we have reported that *P. putida* LPK411 produces three main metabolic products during lupanine fermentation [7]. In this study, we systematically and consistently monitor the transcriptional kinetics of the gene expressing lupanine hydroxylase as well as global genes which may play a critical role during lupanine biodegradation in *P. putida*. We base our hypotheses on the lupanine derivatives’ structures previously observed as well as on the selective catabolism of each enantiomer, which could potentially activate carbon catabolite repression (CCR) mechanisms.

**4. Conclusions**

Deciphering the lupanine biodegradation pathway by a *Pseudomonas* species provides fundamental knowledge of the metabolism during quinolizidine alkaloids biodegradation, which is expected to enhance bioprocess development for the production of high-added value compounds from lupanine wastewater.

**5. Acknowledgements**

This work was supported by the M-ERA.NET collaborative project Biorg4WasteWaterVal+ co-funded by the Research Promotion Foundation (RPF, Cyprus) and Fundação para a Ciência e a Tecnologia (FCT, Portugal).

**References**

1. F. Villalpando-Vargas, L. Medina-Ceja, Seizure 39 (2016) 49-55.
2. M. Erbas, J. Food Qual. 33 (2010) 742-757.
3. F.M.C. Santana, A.M. Fialho, I. Sá-Correia, J.M.A. Empis, J. Ind. Microbiol. 17 (1996) 110-115.
4. M. Koutinas, M. Lam, A. Kiparissides, R. Silva‐Rocha, M. Godinho, A.G. Livingston, E.N. Pistikopoulos, V. De Lorenzo, V.A.P. Martins Dos Santos, A. Mantalaris , Environ. Microb. 12 (2010) 1705-1718.
5. L.A. Nguyen, H. He, C. Pham-Huy, Int. J. Biomed. Sci. 2 (2006) 85-100.
6. D.J. Hopper, M.A. Kaderbhai, Biochim. Biophys. Acta (BBA)-Proteins Proteomics 1647 (2003) 110-115.
7. S. Parmaki, I. Vyrides, M.I. Vasquez, V. Hartman, I. Zacharia, I. Hadjiadamou, C.B.M. Barbeitos, F.C. Ferreira, C.A.M. Afonso, C. Drouza, M. Koutinas, Chemosphere 193 (2018) 50-59.