A novel linuron hydrolase identified by genomic-proteomic analysis of phenylurea herbicide mineralization by *Variovorax* sp. strain SRS16

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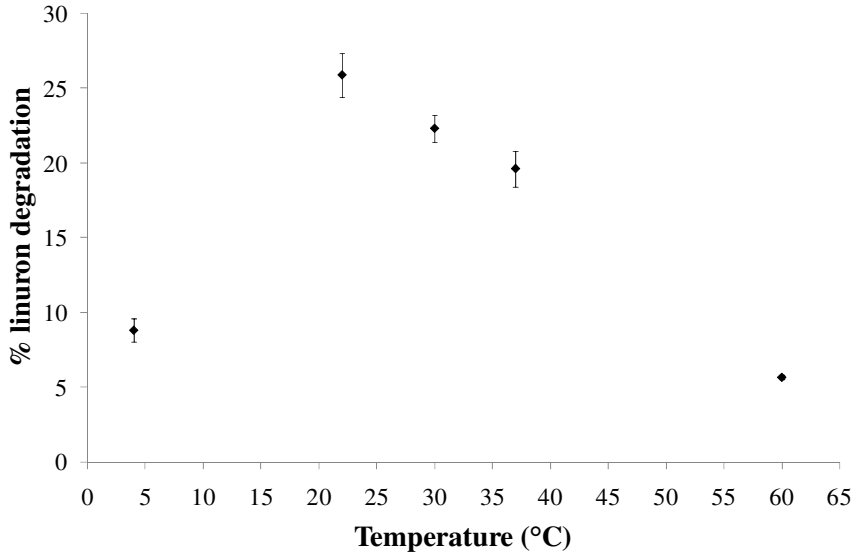
Supplemental material 1

Specification of methodology followed in Southern blotting of linuron/3,4-DCA-degrading bacteria for the presence of *libA*. The hybridization temperature was 62°C and strong, medium and weak hybridization solutions (5XSSC, 0.1% sodium lauroylsarcosine and 0.02% SDS) contained 50, 20 or 0% formamide and 5, 2 and 2% blocking agent respectively. After hybridization the membrane was washed twice in low stringent solution (2 x SSC, 0.1% SDS) (5 min, room temperature) and twice in high stringent solution (0.1 x SSC, 0.1% SDS) (15 min, 68°C). This allowed to detect DNA that was 90, 75, or 60% similar to the gene probe.

Supplementary Table 1 Overview of the PCR primer pairs used in this study and their corresponding loci. The partial amino acid sequences of the amino group transfer protein of the multicomponent aniline dioxygenase in *Variovorax* sp. strains WDL1 (1) and SRS16 (this study) and the amino acid sequences of homologous enzymes in (chloro)aniline degrading Proteobacteria (Genbank accession numbers AAO38206.1, BAD61047.1, ABI20708.1, AAX47239.1, BAA12805.1, BAC82524.1) were used to design primer pair AGT-F/AGT-R. PCR reaction mixtures contained 10 nmol of each dNTP (Invitrogen, Belgium), 25 pmol forward and reverse primer, 1.25 U *Taq* DNA polymerase (Qiagen, Belgium), PCR buffer 1 X (Qiagen, Belgium) and 1 µL of template DNA in a final volume of 50 µL. PCR reaction conditions were an initial denaturation for 5 min at 94°C, 30 cycli consisting of a denaturation step at 94°C for 1 min, an annealing step at 50°C for 1 min and an extension step at 72°C for 1 min, and a final extension step at 72°C for 10 min.

Sequence (5'-3')	Target Gene	Name	Size amplification product (bp)
YTBTCDTGGCCGGAYCARTAYGG CATGTAVAGRTCRGCNARCATCCA	***dcaQ***	ATG-F ATG-R	272
CTCTCATGGCCGGATCAATA TACAGATCGGCCAGCATCCA	***dcaQ***	dcaQ-F dcaQ-R	272
GTACCCGGAGACGAGATCTT ACGCATTCGCCGTGGCTTTT	***dcaT***	dcaT-F dcaT-R	418
GGTGTACTTGGCCCATGAAA TCCAGATAGCGTGTGGCATT	***dcaA$_1$***	dcaA$_1$-F dcaA$_1$-R	392
GTGGCACAACTACTGACACT TCGGCCAGAGTGAAGCTGTA	***dcaA$_2$***	dcaA$_2$-F dcaA$_2$-R	329
TTACCGTCACCGCTGTTTCA GCGTCATACCAGTGGCGTAT	***dcaB***	dcaB-F dcaB-R	511
TACGAAGTTGCAGCCGACAT ACCTGGTCTTGCTCCAGATT	***dcaR***	dcaR-F dcaR-R	347
GCTACGCCACCTTCGTTATT TGTCCGGTTCGTCTCGATAA	***ccdR***	ccdR-F ccdR-R	458
TGGAGGGCCCCTATTTTCTA ACTGTCGACCCACTTCTCAT	***ccdC***	ccdC-F ccdC-R	425
TCAGATTTGGCCGAGGTACT CGTCCTCTGTGTAGTCTTGA	***ccdF***	ccdF-F ccdF-R	380
GTCGATCTGCCTCTCAAGAA AGACCTTCTAGCTGCCACTT	***ccdD***	ccdD-F ccdD-R	677
TGCTGGCACCGGATCTTTAT CCTGCGCTTTGACAGGATAA	***ccdE***	ccdE-F ccdE-R	276
TCCGAGCACCGCAAATACAT ATTCGCATAAAGCGGGCTGT	**ORF 8**	ORF8-F ORF8-R	261
TGGGCTTTCCCGTTTGCTAT CTTCAGCCGGTCTTGTTCAA	**ORF 9**	ORF9-F ORF9-R	177
GATATCAAGCCCGTGACCTT CGCTGAGCTTTCCCTCTTTT	**ORF 10**	ORF10-F ORF10-R	226
TGCTGCAAGCTCTCTTTGCCCT CCAACATCTGACCCCCGGTGTTT	***libA***	LinAmidR-F LinAmidR-R	1571



Supplementary Figure 1 Temperature-dependent activity of linuron hydrolase LibA. All tests were performed in duplicate. The Y-axis shows the percentage of linuron degradation (50 mg L^{-1}) after 1 hour of incubation at the indicated temperature.

References

1. **Breugelmans, P., B. Leroy, K. Bers, W. Dejonghe, R. Wattiez, R. De Mot, and D. Springael. 2010. Proteomic study of linuron and 3,4-dichloroaniline degradation by *Variovorax* sp. WDL1: evidence for the involvement of an aniline dioxygenase-related multicomponent protein. Res. Microbiol. 161:208-218.**