In situ monitoring of linuron degrading *Variovorax* sp. in agricultural soils

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Introduction

Enrichment of linuron degrading organisms from agricultural soils almost exclusively results into the isolation of bacteria belonging to the genus *Variovorax* (Breugelmans, *et al.*, 2007), suggesting that *Variovorax* plays an important role in *in situ* degradation of linuron in agricultural soils. However, it is unclear whether enrichment techniques provide a correct impression of the organisms responsible for *in situ* pesticide degradation (Dunbar, *et al.*, 1997). Monitoring the dynamics of the *Variovorax* population and linuron degradation genes in agricultural soils as a response to linuron supply, will help to determine whether *Variovorax* actually contributes to *in situ* linuron biodegradation in those ecosystems. If this is the case, an increase in the *Variovorax* population size is expected upon exposure to linuron, parallel with an increase in the number of linuron catabolic genes. Therefore, we initiated the development of culture-independent methods to assess the size (real time PCR) and diversity (DGGE) of *Variovorax* in soil. In addition, we started the identification of genes involved in linuron mineralization in *Variovorax* in order to provide sequence information for primer development for PCR monitoring of the catabolic genes.

Development of *Variovorax* specific monitoring methods

The use in PCR of a 16S rRNA gene based *Variovorax*-specific primer set combined with a targeted restriction enzyme digestion of the template DNA before amplification allowed specific recovery of *Variovorax* 16S rRNA gene amplicons from environmental DNA. The restriction enzyme digestion was needed to remove 16S rRNA gene template from the closely related *Acidovorax* genus. The PCR method was optimized as a real-time PCR with a detection limit of 10^5 copies per g of soil. Attachment of a GC-clamp to one of the primers allowed the analysis of the diversity of *Variovorax* by means of Denaturing Gradient Gel Electrophoresis in such a way that different phylogenetic subroups of *Variovorax* could be discriminated. Some of these subgroups currently only consist of linuron-degrading *Variovorax* (Fig. 1).

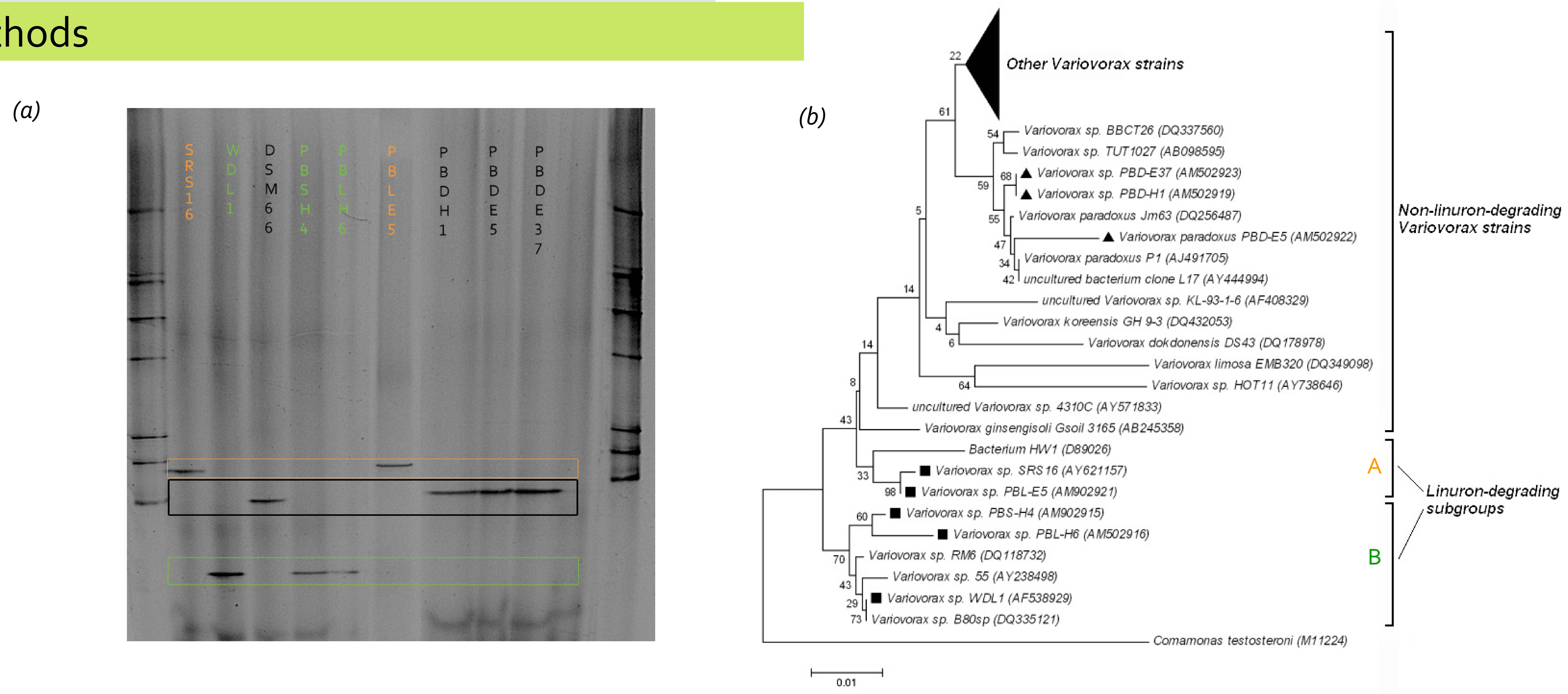

Fig.1: (a) Discrimination of the different phylogenetic subgroups through DGGE. (b) Neighbour-joining phylogenetic tree based on 16SrRNA gene fragments showing the relationship between linuron-degrading and non-linuron-degrading Variovorax sp. (Breugelmans, et al., 2007). Linuron-degrading isolates are indicated with a black square, 3,4-DCA degrading isolates are indicated with a black triangle.

Effect of linuron on the *Variovorax* population and linuron mineralizing activity of an agricultural soil

A preliminary experiment was set-up to study the effect of linuron addition on the linuron mineralizing activity and *Variovorax* population of an agricultural soil. Microcosms filled with an agricultural soil with a history of linuron treatment was subjected to discontinuous irrigation with tap water with or without linuron. At set times, the linuron mineralizing capacity and the size/diversity of the *Variovorax* population was measured by means of ^{14}C-linuron mineralization tests and real time PCR/DGGE, respectively. The lag time before mineralization started in the mineralization assay decreased significantly between time 0 and after two weeks in case linuron was supplied (Fig. 2), suggesting an increase in linuron mineralizing population size. In contrast, no significant change in lag time was observed with samples taken from the microcosms non-fed with linuron. The size of the *Variovorax* population appeared to increase significantly in the soil exposed to linuron (up to 10 X after 2 weeks and up to 100 X after 12 weeks) (Fig. 3) in contrast with the soil non-fed with linuron. Moreover, the supply of linuron seemed to stimulate the growth of a group of *Variovorax*, corresponding to the linuron-degrading subgroup A (Fig.1) of the *Variovorax* cluster (Fig. 4).

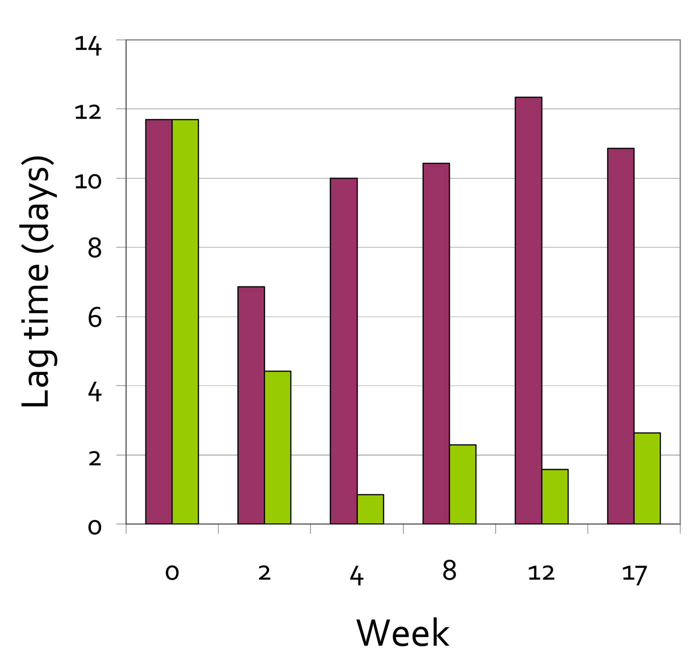

Fig 2: Change in lag time in mineralization kinetics obtained with samples of agricultural soil as a response to irrigation with tap water with or without linuron as determined by ^{14}C-linuron mineralization experiments.

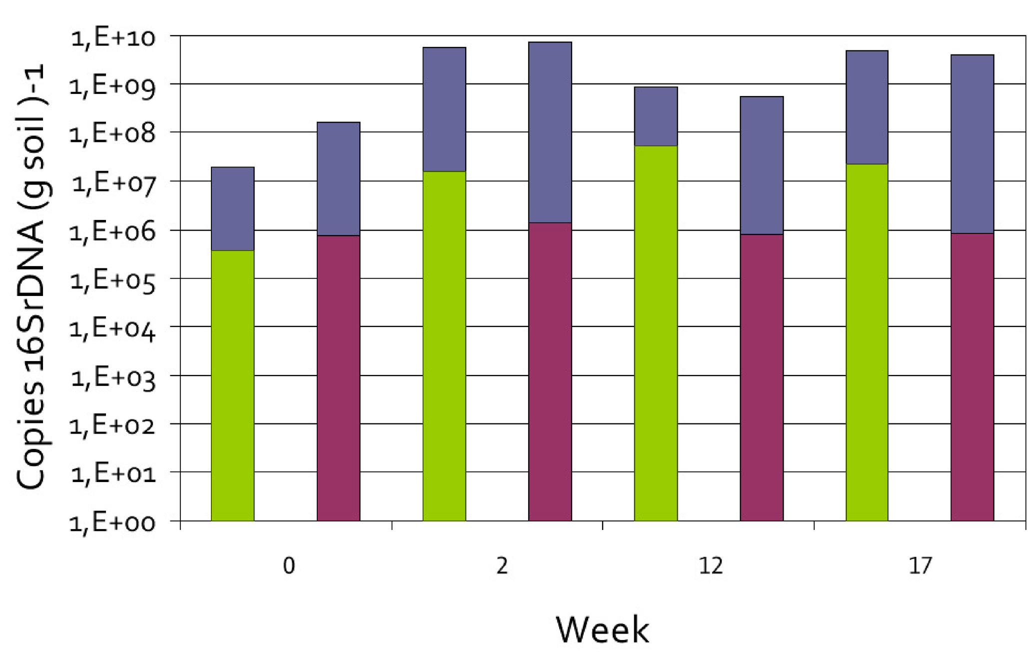

Fig.3: Change in number of Variovorax 16SrRNA gene copies as a response to irrigation with tap water with or without linuron as determined by Variovorax-specific real-time PCR. The number of copies of Variovorax 16SrDNA copies are visualized as part of the total eubacterial number of 16SrRNA gene copies.

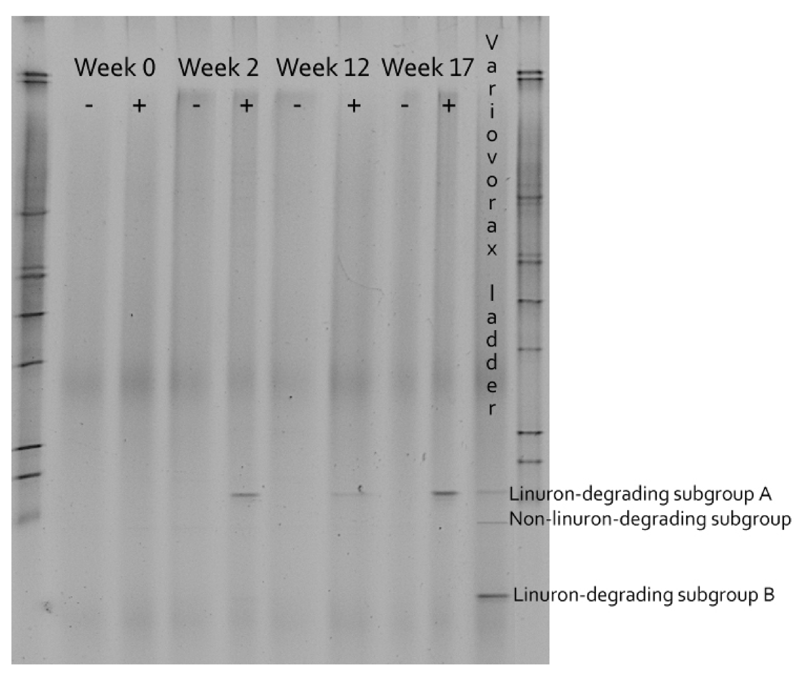

Fig.4: Change in Variovorax diversity as a response to incubation with (+) or without (-) linuron supply as visualized through Variovorax-specific DGGE analysis.

Conclusions

The decrease in lag time of the mineralization capacity of the soil as a response to linuron supply indicates the stimulation of the linuron degrading population. The parallel increase of the *Variovorax* population size and the stimulation of a specific group of *Variovorax* as a response to the linuron supply suggests the *in situ* involvement of *Variovorax* in linuron mineralization.

References

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