Interactions between pesticides and microorganisms: The case of biodegradation of synthetic ß-triketone herbicides

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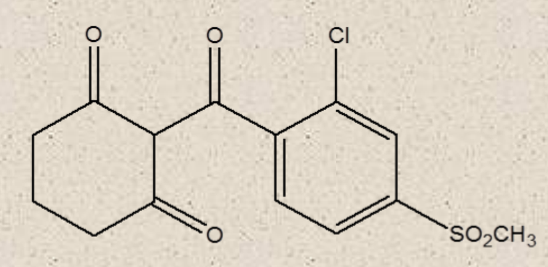
Introduction

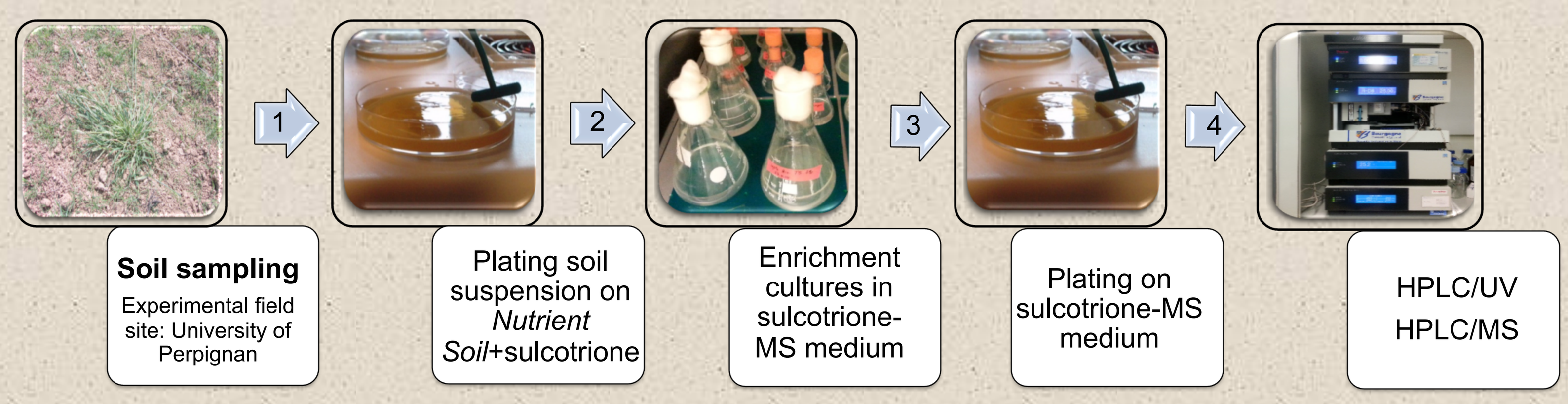
Agricultural use of pesticides ensures a higher crop quality and production but it is also one of the major sources of diffuse pollution in the environment. Microbial degradation is considered as an important dissipation process limiting the accumulation of pesticides in the environment. In this context, two bacterial strains able to degrade sulcotrione, a β-triketone herbicide, were isolated from an agricultural soil previously exposed to this herbicide.



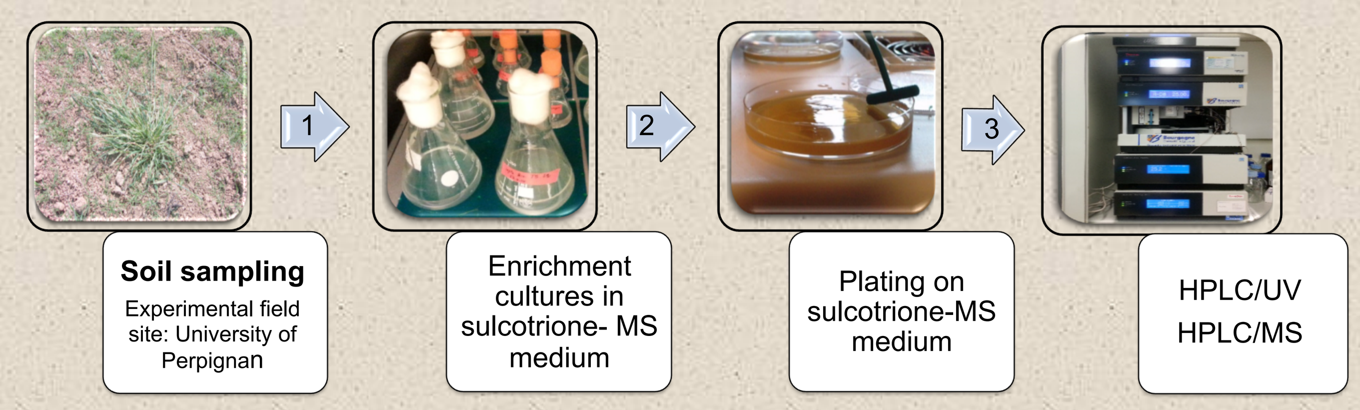
sulcotrione

Bacterial isolation procedure: one soil, two strategies

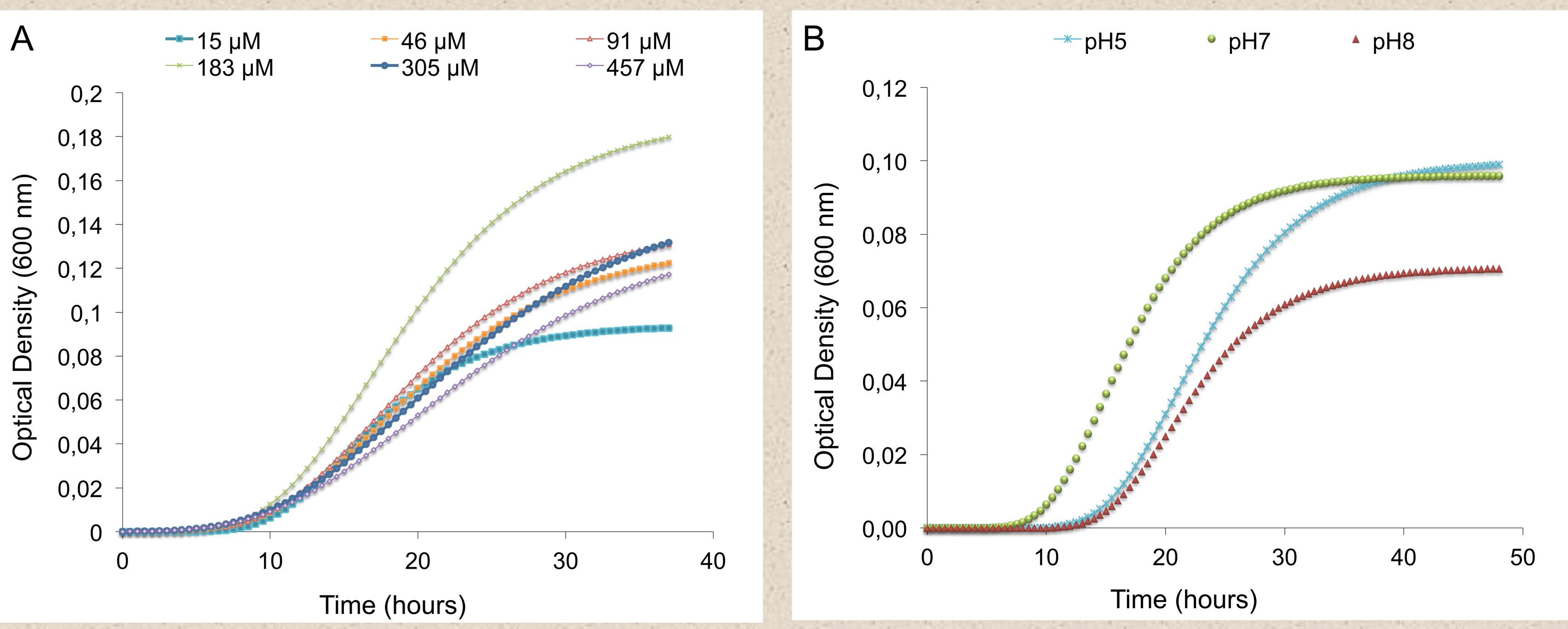
STRATEGY A



STRATEGY B



Characterization of *Pseudomonas* sp. 1OP, a sulcotrione-degrading bacteria (Strategy A)

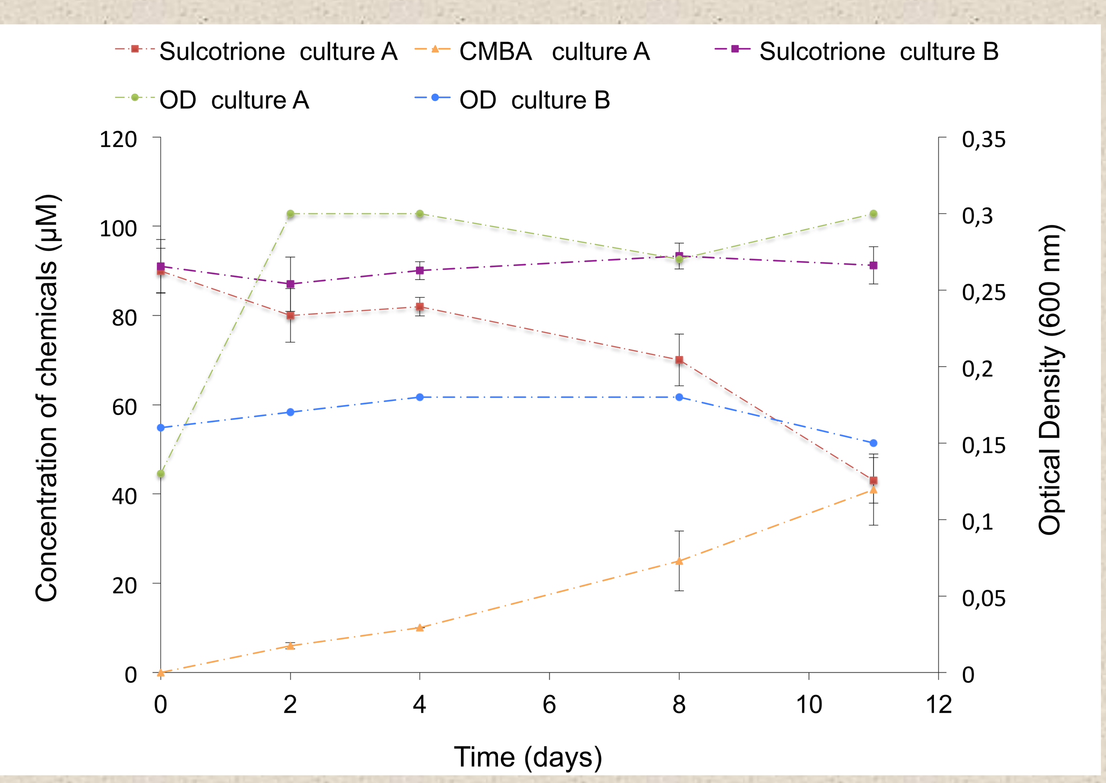


(A) Growth monitoring of strain *Pseudomonas sp.*1OP *vs* different concentrations of sulcotrione in MSM (mean values) fitted to the modified Gompertz model. **(B)** Effect of initial pH on growth performances of the isolate 1OP fitted to the modified Gompertz model.

	Concentration of the herbicide in liquid medium		μ_{max} (h^{-1})	λ (h)	G (h)	OD_{max} (a.u.)
MSM + sulcotrione	15 µM		0.0078 ± 0.001	9	89.0	0.096 ± 0.015
	46 µM		0.0082 ± 0.001	9	84.5	0.130 ± 0.015
	91 µM		0.0097 ± 0.001	9	71.0	0.137 ± 0.011
	183 µM		0.0120 ± 0.001	9	55.0	0.180 ± 0.020
	305 µM		0.0090 ± 0.001	9	77.0	0.140 ± 0.021
	457 µM		0.0089 ± 0.001	9	78.0	0.130 ± 0.022
TS	–	1st phase[a]	0.1050 ± 0.0024	5	7.0	–
	–	2nd phase[a]	0.2779 ± 0.0175	5	2.5	1.560 ± 0.015

[a] The two phases observed correspond to a diauxic growth on a rich medium (TS).

Growth parameters of the isolate *Pseudomonas sp.*1OP. The maximum specific growth rate (μ_{max}), the lag time (λ), the generation time (G) and the maximum optical density (OD_{max}) values were determined for MSM+sulcotrione and TS media.



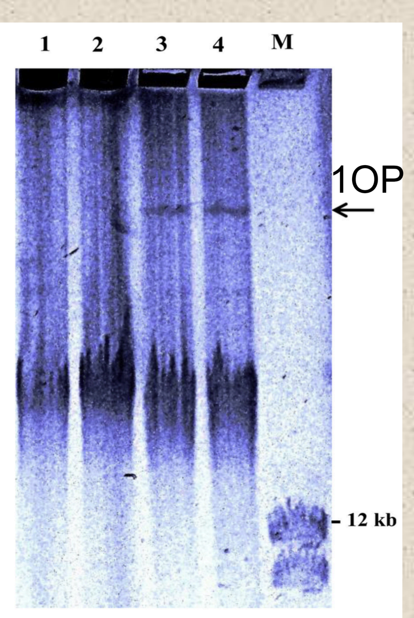
Degradation kinetics of sulcotrione, accumulation of CMBA and growth measurement of *Pseudomonas* sp.1OP.

Culture A (MSM+sulcotrione) *vs* **culture B** (10 repeated transfers from rich medium and then placed in MSM+sulcotrione).

No degradation was observed neither with CMBA nor tembotrione, another herbicide of the ß-triketone family.

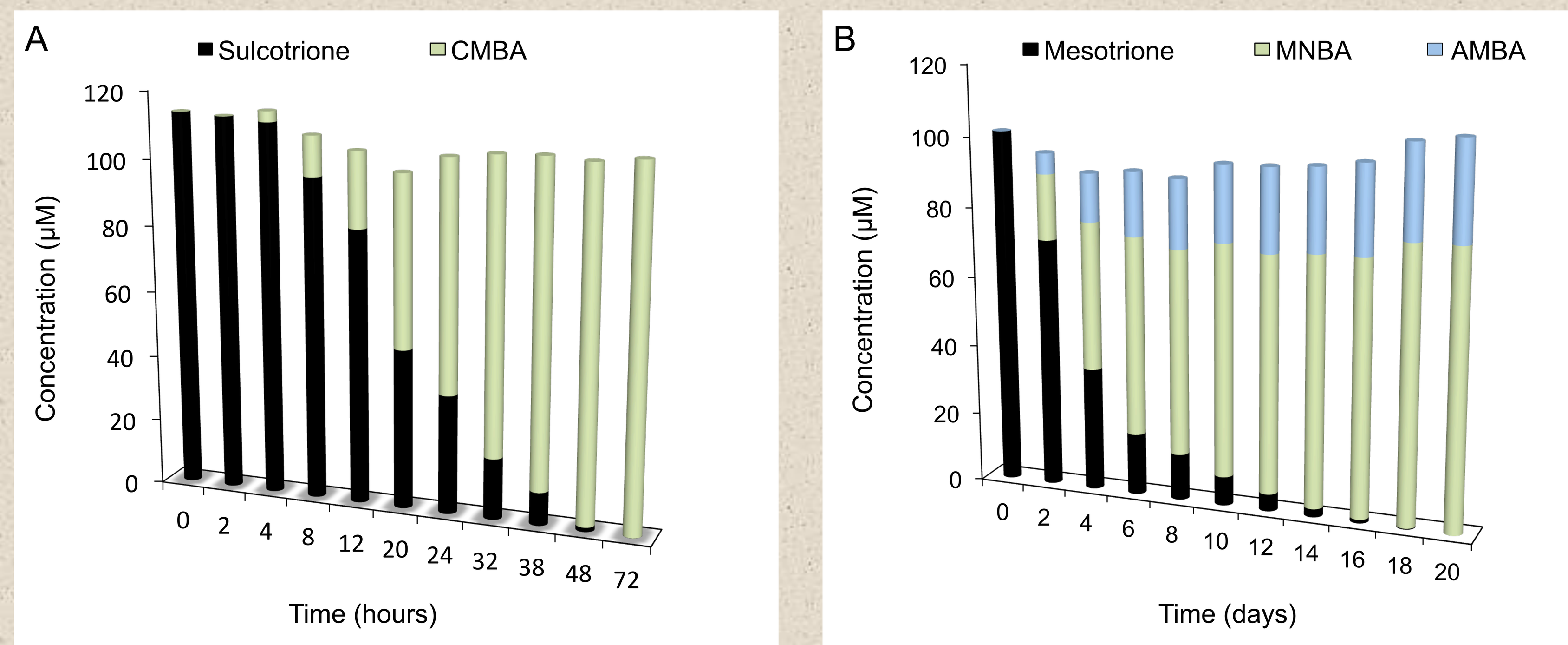
Genetic localization of sulcotrione degradation in Pseudomonas sp. 1OP

Plasmid profiles from *Pseudomonas sp.* 1OP obtained in MSM+sulcotrione or in absence of sulcotrione in a rich medium revealed the presence of a >12 kb plasmid. **1OP plasmid was eliminated by curing experiment carried out on rich medium and sulcotrione-degrading ability was lost without pesticide selection pressure.**



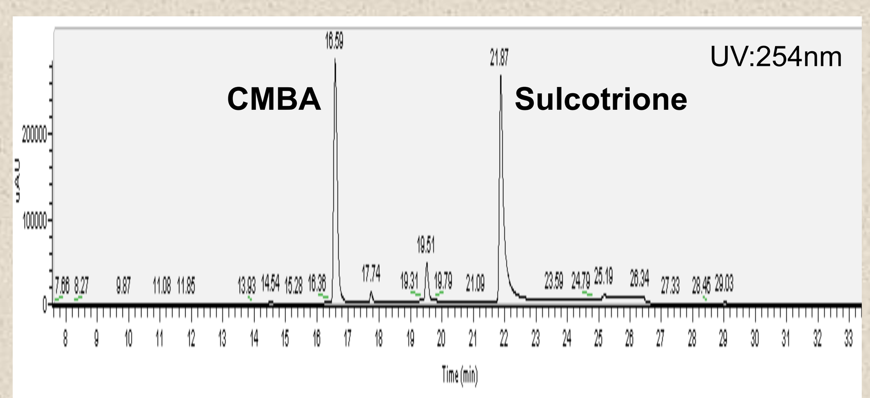
Lanes 1 and 2: strain cultivated in absence of sulcotrione in rich media. Lanes 3 and 4: strain cultivated in MSM in presence of sulcotrione (91 mM). Position of the plasmid is indicated by arrow.

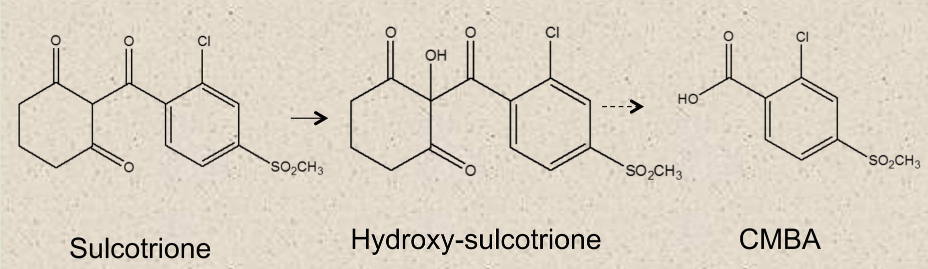
Characterization of *Bradyrhizobium* sp. SR1, a sulcotrione and mesotrione degrading bacteria (Strategy B)



Degradation kinetics of **(A)** sulcotrione and **(B)** mesotrione by Bradyrhizobium sp. SR1 in resting cell experiments. Cumulative formation of metabolites during sulcotrione (CMBA) and mesotrione degradation is represented.

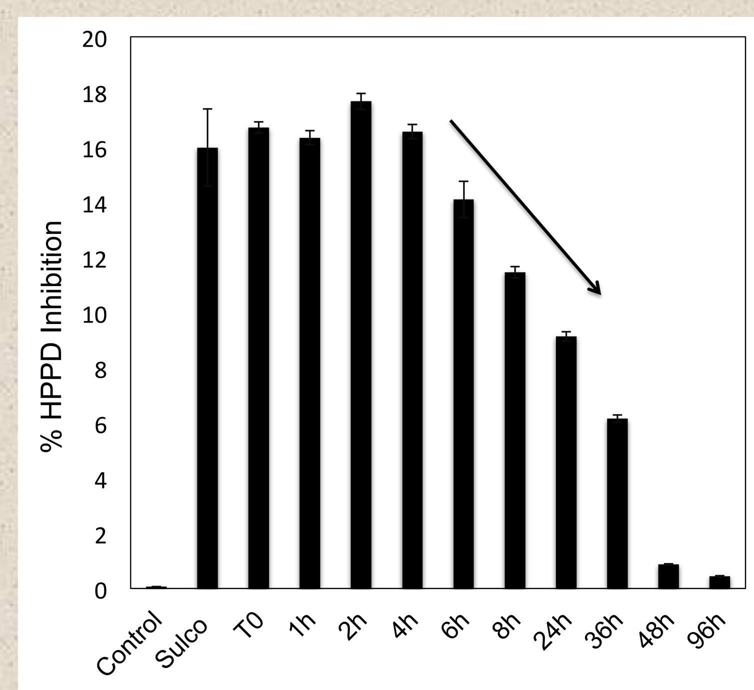
Study of sulcotrione metabolites





Concomitantly with the appearance of CMBA, a new metabolite in *Bradyrhizobium sp.* SR1 culture medium was detected and identified as hydroxy-sulcotrione, an intermediate metabolite of sulcotrione that finally gives CMBA.

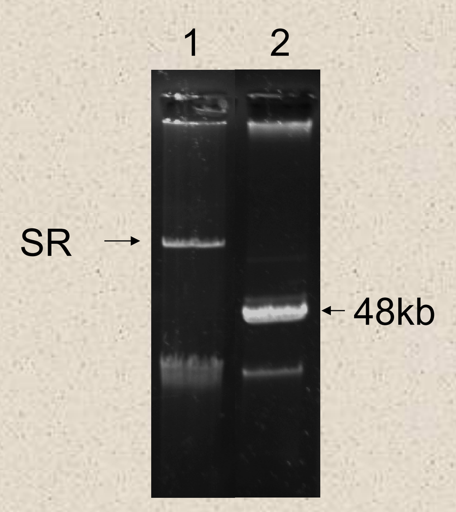
Microbial toxicity of sulcotrione and mesotrione metabolites



The toxicity of triketone molecules and their related metabolites was estimated by monitoring 4-hydroxyphenylpyruvate dioxygenase inhibition (HPPD). **Toxicity due to HPPD inhibition was mainly linked to parent molecules, and not to the formed metabolites.**

Genetic localization of sulcotrione degradation in Bradyrhizobium sp. SR1

Plasmid profiles from (1) *Bradyrhizobium sp.* SR1. The size of the plasmid is indicated in kb (2) *Sphingomonas sp.* SH., used as molecular weight marker.
SR1 plasmid was not eliminated by curing experiment carried out on rich medium and that sulcotrione-degrading ability was maintained without pesticide selection pressure.



pSR1 profil (>48kb)

Conclusion: Two degrading strains exhibiting different genetic features

- ***Pseudomonas sp.*1OP** was the first bacterial strain described in the literature as capable of degrading sulcotrione to 2-chloro-4-mesylbenzoic acid (CMBA). Its growth performances have shown that initial neutral pH conditions and 183 µM of sulcotrione seemed to be the best cultural environment for this strain. The isolate harboured a catabolic plasmid involved in sulcotrione biodegradation process.
- ***Bradyrhizobium sp.* SR1** was able to biotransform two ß-triketone herbicides, sulcotrione and mesotrione. The dissipation of sulcotrione and mesotrione led to the accumulation of different known metabolites, CMBA and MNBA/AMBA. A 14 000 Tn*5* mutant library was constructed using a Tn*5* mutagenesis approach conducted on *Bradyrhizobium sp.* SR1. Full sequencing of mutants are ongoing to identify possible degrading gene candidates.