In vivo metabolism analysis in *Escherichia coli* to elucidate substrate scope of herbicide metabolizing CYP81As from crops and weeds

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Introduction

In agriculture, herbicides are an important weed control method. Most herbicides selectivity target weeds, but leave crops unaffected. One such selectivity mechanism is herbicide detoxification, mediated by cytochrome P450s (P450s). This enzyme superfamily is heme proteins which functions as monooxygenases. Plant genomes contain hundreds of *P450* genes, with most involved in the synthesis of plant specialized metabolites or plant hormones.

Recently, it was reported that the CYP81A subfamily was involved in the herbicide detoxification of *Poaceae*. In rice, CYP81A6 was involved in the detoxification of multiple herbicides with different mode of actions, and in different chemical groups. For example, bensulfuron-methyl (BSM), which is a typical sulfonylurea herbicide, is rapidly detoxified to the non-phytotoxic compound, *O*-demethylated BSM by CYP81A6. In contrast, BSM detoxification in herbicide-sensitive weeds, such as late watergrass (*Echinochloa phyllopogon*), a noxious weed of paddy rice fields, is slower than rice. This is why rice is highly tolerant to BSM, but weeds are sensitive to the compound (1).

However, significant herbicide selection pressures have led to the emergence of herbicide-resistant weeds, seriously threatening agriculture. Of the resistance mechanisms, the improvement of detoxification metabolism function, also called metabolism-based herbicide resistance (MHR) is the most threatening. Because MHR often exhibits resistance to herbicides with different mode of actions, even yet-to-be discovered herbicides (2). For example, late watergrass populations in California exhibit resistance to multiple herbicides from different chemical groups, with different modes of action. The MHR in the weed is endowed by *CYP81A12* and *CYP81A21* gene overexpression, when compared to herbicides sensitive population (3). However, herbicide selectivity for CYP81As in rice and late watergrass has not been elucidated.

In this study, *CYP81A*s from rice and late watergrass were heterologously expressed in *Escherichia coli*. We then compared their herbicide metabolic patterns using these *E. coli* harboring *CYP81A* strains.

Material and methods

Measurement of herbicide metabolic rates using CYP81A transgenic bacteria

E. coli BL21 (DE3) cells were transformed using pET28(a)+ plasmids carrying *CYP81A*s. The transformants were cultured at 34°C for 48 h in casamino acid based autoinduction medium containing herbicides. After culturing, residual herbicides in the medium were qualified by high performance liquid chromatography to measure herbicide metabolic rates, and compare the metabolic patterns of each herbicide with each CYP81As.

Result and discussion

In this study, the metabolic capacity of several CYP81As for various herbicides was evaluated. Our herbicide metabolism studies using *E. coli* harboring *CYP81A*s showed that CYP81As exhibited differences in herbicide metabolizing selectivity. Detailed results will be reported at the presentation. These data will help select efficient herbicides for MHR weeds.

References

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