

# Characterization and molecular mechanism of a naturally occurring metsulfuron-methyl resistant strain of *Pseudomonas aeruginosa*

Xing Huang · Jian He · Xiao-Fei Sun · Ji-Quan Sun ·  
Yong-Feng Li · Jing-Jing Shen · Shun-Peng Li

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**Abstract** Strain L36, naturally resistant to the herbicide metsulfuron-methyl (SM), was isolated and characterized with respect to the molecular mechanism of resistance. The isolate was identified as *Pseudomonas aeruginosa* based on bacterial morphology, physiology, cellular fatty acid, and 16S rRNA gene sequence. Minimal inhibitory concentrations of metsulfuron-methyl against the growth of L36 and wild type isolate PAO1 were 6.03 and 1.33 mM, respectively. L36 carried a nucleotide base change in the acetolactate synthase (ALS) gene that coded for a single amino acid mutation (Ala29 → Val29). The mutated *ilvIH* gene was functionally expressed, purified, and the kinetic properties of the purified ALS were tested. The mutant enzyme had  $K_m$  for pyruvate fourfold higher than the wild type enzyme, and  $K_i^{APP}$  for sulfonylureas some 30-fold higher. The A29 V mutation in the ALS resulted in the resistance of *P. aeruginosa* to sulfonylurea herbicides but not to imidazolinone herbicides.

**Keywords** Acetolactate synthase · Metsulfuron-methyl · *Pseudomonas aeruginosa* · Resistance mechanism

## Introduction

Acetolactate synthase (ALS, EC 4.1.3.18), also named acetoxyacid synthase (AHAS), is the first common

enzyme in the biosynthetic pathway leading to the branched amino acids valine, leucine and isoleucine in plants, bacteria and yeast (Blair and Martin 1988; Brown 1990; Tan and Medd 2002). ALS has dual catalytic functions: it catalyzes the condensation of 2-acetolactate from two molecules of pyruvate in the first step of the valine and leucine synthetic pathways, and also catalyzes the formation of 2-aceto-2-hydroxybutyrate from pyruvate and 2-ketobutyrate in the second step of isoleucine biosynthesis (Chipman et al. 2005). ALS requires three cofactors for its catalytic activity, thiamin pyrophosphate (TPP), flavin adenine dinucleotide (FAD), and a divalent metal ion,  $Mg^{2+}$  (Pang et al. 2002). In *Escherichia coli* and *Salmonella typhimurium*, at least three active ALS isozymes have been identified, namely ALS I, II, and III encoded within the *ilvBN* (Wek et al. 1985), *ilvGM* (Sutton and Freundlich 1980) and *ilvIH* (Squires et al. 1983) operons respectively, and ALS is a tetramer composed of two large and two small subunits (Schloss et al. 1985). However, some prokaryotes synthesize a single ALS, e.g. only *ilvIH* was found in the genomes of the genus *Pseudomonas* (Chipman et al. 1998; Stover et al. 2000), and ALS is composed of one large catalytic and one small regulatory subunit in *P. aeruginosa* (Arfin and Koziell 1973).

The branched amino acids are synthesized by plants and bacteria, but not by animals. Therefore, the ALS of the biosynthetic pathway is potential target for the development of herbicides. Indeed, some of herbicides used worldwide over the past 20 years act by inhibiting ALS. However, the repeated use of herbicides in the field has resulted in weeds developing resistance to several ALS-inhibiting herbicides. Herbicide-resistant mutations of ALS in plants, yeast and bacteria have been obtained, including both naturally resistant isolates under field conditions and those that have been introduced intentionally in the

X. Huang · J. He · X.-F. Sun · J.-Q. Sun · Y.-F. Li · J.-J. Shen · S.-P. Li (✉)

Key Laboratory of Microbiological Engineering of Agricultural Environment, Ministry of Agriculture, College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, People's Republic of China  
e-mail: lsp@njau.edu.cn

laboratory (Haughn and Somerville 1986; Yoon et al. 2002; Duggleby et al. 2003). In most cases, resistance is due to a single point mutation that alters the structure of the ALS, making it less sensitive to the herbicides. Some of these resistant-mutation sites have been identified in plants, yeast and bacteria, e.g. resistant-mutants have been reported in tobacco (A121T, Chong and Choi 2000), cocklebur (*Xanthium* sp.) (W552L, Bernasconi et al. 1995), cotton (W563CS, Rajasekaran et al. 1996), *Arabidopsis thaliana* (S653 N, Chang and Duggleby 1998), and *E. coli* (W464AFLQY, Ibdah et al. 1996).

To establish the molecular mechanism of resistance in *Pseudomonas aeruginosa* to metsulfuron-methyl, the SM-resistant strain L36 was isolated from soils previously impacted by metsulfuron-methyl spills. The mutated *ilvIH* gene was cloned, functionally expressed, purified, and the kinetic properties of the purified ALS were tested. The result revealed important clues about how mutation led to herbicide resistance.

## Materials and methods

### Chemicals and medium

Soil samples were collected from the surface layer (0–10 cm) from Huarui Agrochemical Factory (Jiangsu, China). Metsulfuron-methyl (98.0% purity) and chlorsulfuron (99.0% purity) were purchased from Changzhou Agrochemical Factory (Jiangsu, China). Imazethapyr (98.0% purity) was purchased from Xinnongji Agrochemical Company (Shangong, China). Imazaquin (96.0% purity) was purchased from Shandong Cynda Chemical Co., Ltd (Shandong, China). Other chemicals used were analytical grade.

The mineral salt medium (MSM) had the following composition (per liter): NaCl, 1.0 g;  $\text{NH}_4\text{NO}_3$ , 1.0 g;  $\text{K}_2\text{HPO}_4$ , 1.5 g;  $\text{KH}_2\text{PO}_4$ , 0.5 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g;  $\text{FeSO}_4$ , 0.025 g; trace element solution 10 ml (Ferrari et al. 1994); pH7.0. MSGM medium was MSM supplement with glucose at a final concentration of  $100 \text{ mg l}^{-1}$ . Luria-Bertani (LB) medium contained bactotryptone ( $10.0 \text{ g l}^{-1}$ ), yeast extract ( $5.0 \text{ g l}^{-1}$ ), NaCl ( $5.0 \text{ g l}^{-1}$ ) (pH 7.0). For solid medium, agar was added to a final concentration of 1.5% (w/v).

### Isolation and identification of SM-resistant strain

About 10.0 g of soil sample was added to Erlenmeyer flasks (250-ml) containing 100 ml sterilized water incubated at 30°C on a rotary shaker at  $200 \text{ rev min}^{-1}$  for 30 min. The mixed cultures were serially diluted and spread onto MSGM agar plate containing different

concentrations of metsulfuron-methyl (0, 0.66, 1.31, 2.62, 3.93, 5.24 mM). After incubation at 30°C for 2 days, visible colonies were picked and repeatedly streaked onto MSGM agar containing metsulfuron-methyl until pure cultures were obtained. After repeated streaking, a high-level resistant strain, designated as L36, was selected for further research.

The identification of L36 was carried out according to *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994). To determine the fatty acid composition, cultures of L36 and *P. aeruginosa* wild type strain PAO1 were grown in 100 ml LB medium at 30°C for 24 h. Cells were harvested, and the pellet was washed three times with distilled water. The fatty acids in the bacterial cells were saponified, methylated, and extracted as described previously (Zhu et al. 2005). The resulting methyl ester mixtures were separated by gas chromatograph. Fatty acids were identified by the standard MIDI Microbial Identification system (Wei et al. 2008).

The genomic DNA was extracted by the method of high-salt concentration precipitation. The 16S rRNA gene was amplified by PCR using standard procedures (Lane 1991), and the sequence was deposited in GenBank (accession number DQ989017). The sequence was compared to known sequences found in the Ribosomal Database Project. Multiple alignments were carried out using CLUSTALX 1.8.3, and phylogenesis was analysed using MEGA (version 3.0) software. The distance was calculated using the Kimura two-parameter model. An unrooted tree was built using the neighbour-joining method.

### Sensitivity tests

The sensitivities of L36 and PAO1 to different ALS-inhibiting herbicides (metsulfuron-methyl, chlorsulfuron, imazethapyr and imazaquin) were determined by minimal inhibition concentration (MIC) test. The MIC was defined as the lowest concentration of herbicides that would inhibit visible bacterial growth after incubation. L36 and PAO1 were streaked onto MSGM agar plate containing different concentration of ALS-inhibiting herbicides, and a control included no herbicides. The plates were incubated at 30°C for 24 h. After incubation, the visible bacterial growth was observed, and the MIC was determined.

### Cloning, expression and purification of ALS

The *ilvIH* gene from L36 and PAO1 was cloned using PCR-based technique. The primers were designed as follows: 5'-GTGGAGCTTTTATCTGGC-3' and 5'-TCAGATGCTCAGAGTCTTGT-3'. PCR reactions were carried out with a PTC 200 gradient cycler (MJ Research, Waltham, MA, 02451-2173) under the following conditions: 3 min at 95°C;

30 cycles of 30 s at 94°C, 30 s at 52°C and 1 min at 72°C; plus an additional 10 min cycle at 72°C. PCR fragments were ligated into the linear vector pMD18-T (TaKaRa Biotechnology) after purification by agarose gel electrophoresis, and then transformed into competent *E. coli* DH5a cells. The recombinant plasmid in positive clones was extracted and used as the template for direct sequencing of the *ilvIH* fragment by using an automatic sequencer (Applied Biosystems, model 3730). Manipulation and editing of nucleic acids and protein sequences of ALS were made using the BioEdit program.

The DNA fragments encoding the open reading frames of ALS from L36 and PAO1 were amplified by PCR using the gene specific primers 5'-AGCTGGATCCGTGGAGC TTTTATCTGGC-3' (*Bam*HI) and 5'-AGCTCTCGAG TCAGATGCTCAGAGTCTTGT-3' (*Xho*I). The amplified DNA was inserted into the *Bam*HI/*Xho*I sites of pET29a. Recombinant expression plasmid (pET-IH) was transformed into *E. coli* BL21 (DE3). Transformed bacteria were grown at 37°C in LB medium containing 30 µg ml<sup>-1</sup> kanamycin until optical density of the culture at 600 nm reached 0.5. Expression of recombinant ALS was induced by addition of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and additional incubation for 4 h at 37°C. Cells expressing fusion protein were harvested, centrifuged, and washed with 20 mM potassium phosphate buffer (pH 7.0). After the cells were disrupted by ultrasonic treatment for a total of 10 min in ice, insoluble material was removed by centrifugation (12,000g for 40 min at 4°C), and the supernatant was passed through a 0.45 µm membrane filter for purification.

The ALS expressed from pET29a(+) had a C-terminal 6× His tag, and the histidine-tagged ALS (IlvIH) were purified in their native forms by affinity chromatography on a Ni-NTA Agarose column (Qiagen, Shanghai, China) using the method described previously (Engel et al. 2004). The purity of the proteins obtained was assessed by SDS/PAGE and the protein concentration was determined with bovine serum albumin (Sigma, Shanghai, China) as the standard protein.

#### Enzyme assay

Enzyme activities of the purified ALS were measured using the standard colorimetric assay for acetoin as previously described (Yoon et al. 2002) with slight modification. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.5), 1 mM TPP, 10 mM MgCl<sub>2</sub>, 20 µM FAD, 100 mM pyruvate, and the enzyme in the absence or presence of different concentrations of inhibitors. The reaction was started by adding the enzyme solution into the buffer containing substrate and cofactors at 37°C and terminated after adding 6 M H<sub>2</sub>SO<sub>4</sub>. The reaction product

acetolactate was allowed to decarboxylate, and the acetoin formed by acidification was measured with 0.5% creatine and 5% α-naphthol. The absorbance of the reaction mixture was detected at 525 nm. One unit (U) of activity was defined as the formation of 1 µmol acetolactate per minute, and the specific activity was expressed as U mg<sup>-1</sup> protein under standard conditions.

The kinetic characteristics of the resistant and wild-type enzyme were studied, including  $K_m$  for pyruvate,  $K_c$  for FAD, TPP and  $K_i^{app}$  of four herbicides, metsulfuron-methyl, chlorsulfuron, imazethapyr and imazaquin. The value of  $K_m$  for the substrate were determined by fitting the data to Eq. 1, and the values of activation constant ( $K_c$ ) for cofactor were obtained by fitting the data into Eq. 2, by the non-linear least-squares and simplex method for error minimization.

$$v = V_{max}/(1 + K_m/(S)) \quad (1)$$

$$v = V_0 + V_{max}/(1 + K_c/(C)) \quad (2)$$

In these equations,  $v$  is the reaction velocity,  $V_{max}$  is the maximum velocity,  $V_0$  is the activity without adding cofactors,  $K_m$  is the Michaelis–Menten constant,  $K_c$  is the activation constant, ( $S$ ) is substrate concentration, and ( $C$ ) is added cofactor concentration. The  $K_i^{app}$  values were determined by fitting the data into Eq. (3).

$$v_i = V_0/(1 + (I)/K_i^{app}) \quad (3)$$

In this equation,  $v_i$  and  $v_0$  represent the rates in the presence or absence of the inhibitor, respectively, and ( $I$ ) is the concentration of the inhibitor. The  $K_i^{app}$  is the apparent  $K_i$ , which is the concentration of the inhibitor resulting in 50% inhibition under standard assay conditions, which is also known as IC<sub>50</sub>.

## Results

### Isolation and identification of the SM-resistant strain L36

After serial dilution and repeated streaking, a high-level resistant strain, designated as L36, was selected for further research. Strain L36 was a Gram-negative, short, rod-shaped bacterium and formed aerogenous colony on LB agar medium. Biochemically, it revealed positive results for oxidase, catalase, gelatin liquefaction, nitrate reduction and utilized fructose, sucrose, galactose, xylose, mannitol as sole carbon sources. Negative results were observed in the hydrolysis of starch, Voges-Proskauer test and methyl red test. The physiological and biochemical characteristics of L36 showed no difference from the results of strain PAO1.

The cellular fatty acid composition of L36 and PAO1 were analysed, and the results revealed that the fatty acids of L36 were extremely similar to PAO1. The most abundant straight-chain fatty acids in two strains were hexadecanoic acid (16:0), hexadecenoic acid (16:1), and octadecenoic acid (18:1). Each of these strains also contained relatively large amounts of 3-OH 10:0, 2-OH 12:0, and 3-OH 12:0, and none of these strains contained 3-hydroxytetradecanoic acid (3-OH 14:0).

The sequence of the 1.5 kb 16S rRNA gene of L36 was deposited in the GenBank database under accession number DQ989017. Multiple alignments revealed that the 16S rRNA gene sequence of L36 was closely related to that of *P. aeruginosa* strain DSM50071 (98.4% of similarity, Toschka et al. 1988) (Fig. 1). According to its morphology, physiology, cellular fatty acid, and 16S rRNA gene sequence, L36 was identified as *P. aeruginosa*.

Herbicide resistance

The resistance of L36 and PAO1 to different ALS-inhibiting herbicides was determined. MICs of L36 to metsulfuron-methyl and chlorsulfuron on MSGM plates were 6.03 and 5.48 mM respectively, whereas for PAO1 it was 1.33 and 1.12 mM respectively. The result showed strain L36 was resistant to the sulfonylureas metsulfuron-methyl and chlorsulfuron. Strain L36 was also found sensitive to imidazolinones, another ALS-inhibitor herbicide, which is structurally unrelated to sulfonylureas. There was no

difference between L36 and PAO1 in the sensitivity to imazethapyr and imazaquin, implying that the sulfonylureas resistant strain L36 had no cross-resistance to imidazolinones.

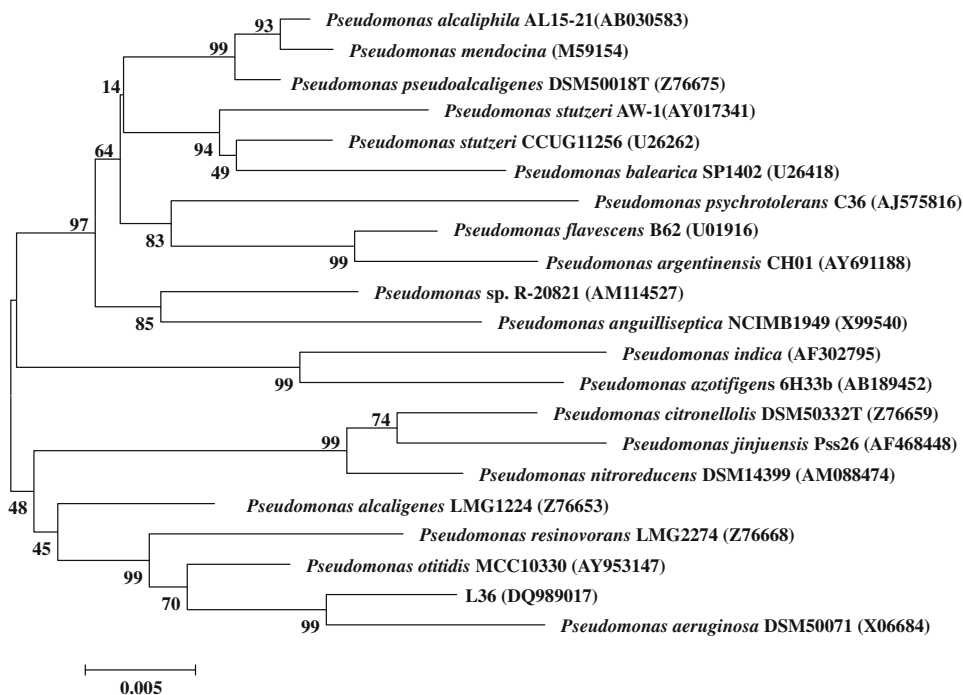
PCR amplification of ALS gene

The nucleotide sequences of the *ilvH* and *ilvI* genes of L36 were obtained by PCR amplification and deposited as EF105336 and EF105338 in GenBank. The amino acid sequence of IlvH subunit of L36 was identical to that of PAO1. But a mutation (C-to-T) at nucleotide 86 of the *ilvI* gene was found in L36, and this mutation resulted in Ala<sub>29</sub> (GCC) being changed to Val<sub>29</sub> (GTC) in the amino acid sequence of the IlvI subunit. In comparison of the amino acid sequence of IlvI among different species of bacteria, the composition of Ala<sub>29</sub> from *P. aeruginosa* is remarkable in a highly conserved domain which was located near the N-terminus (Fig. 2). A29 V has been described previously as the equivalent mutation of A26 V in *E. coli* ALS II and

<i>E.coli</i>	QVFGYPGGAVLDIYDALHT
<i>E.coli</i> mutation	-----V-----
Yeast	TVFGYPGGAILPVYDAIHN
Yeast mutation	-----V-----
<i>P.aeruginosa</i> PAO1	YIYGYPGGALLHIYDALFK
<i>P.aeruginosa</i> L36	-----V-----

Fig. 2 Comparison of the partial amino-acid sequences of *P. aeruginosa* and the equivalent mutation known to confer resistance to metsulfuron-methyl in *E. coli* and yeast

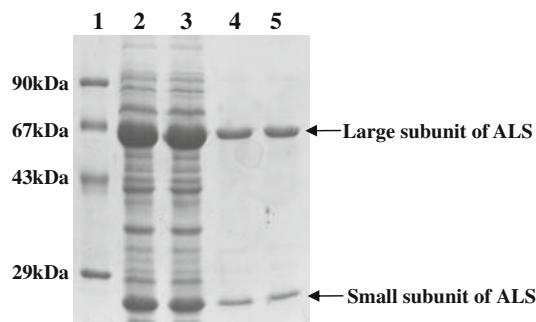
Fig. 1 Phylogenetic tree constructed by the neighbor-joining method based on 16S rRNA gene sequences of L36 and related bacteria. Bootstrap values, expressed as percentages of 1,000 replications, are given at branching points. The scale bars represent 0.005 substitutions per site



mutation of A117 V in yeast ALS. In *E. coli* ALS II, the A26 V mutation resulted in reduced levels of ALS and reduced sensitivity to metsulfuron-methyl (Yadav et al. 1986). In yeast ALS, the A117 V mutation exhibited rather specific resistance to metsulfuron-methyl (Duggleby et al. 2003). But different from the reported equivalent mutations, the naturally resistant strain L36 was obtained from an ecosystem contaminated with metsulfuron-methyl runoff and was not induced in the laboratory.

#### Expression and purification of ALS

The *ilvHI* genes of L36 and PAO1 were overexpressed from *E. coli* BL21 (DE3). SDS-PAGE of samples of the transformed bacteria before and after induction of expression of the ALS with IPTG showed enhancement bands of 60 kDa and 20 kDa, the expected size of the large catalytic and small regulatory subunits of *P. aeruginosa* ALS. The overexpressed protein was purified and two subunits with rather high degree of purity were obtained (Fig. 3). The purified ALS of L36 and PAO1 were named as mutant ALS (mALS) and wild ALS (wALS) respectively.



**Fig. 3** SDS-PAGE analyses of ALS from *P. aeruginosa*. Lane 1: protein molecular marker; Lane 2: overexpressed mALS; Lane 3: overexpressed wALS; Lane 4: purified mALS; Lane 5: purified wALS

#### Kinetic properties of mALS and wALS

Mutant and wild enzymes were characterized in term of kinetic parameters, including  $K_m$  for pyruvate,  $K_c$  for FAD, TPP and  $K_i^{app}$  of four herbicides. The specific activity of the purified enzymes was determined. The activity of mALS showed no significant difference from the wALS. The  $K_m$  value of mALS for pyruvate was 12.54 mM which was approximately fourfold higher than the corresponding value for wALS (Table 1). However, the  $K_c$  values of mALS for FAD and TPP were marginally different from those of wALS.

The activities of wALS and mALS were assayed in the presence of different herbicide inhibitors. The herbicides metsulfuron-methyl, chlorsulfuron, imazethapyr and imazaquin were inhibitors of wALS with  $K_i^{app}$  values of 0.29  $\mu$ M, 1.04  $\mu$ M, 0.93 mM and 0.83 mM, respectively (Table 2). The mALS was strongly resistant to both metsulfuron-methyl and chlorsulfuron with  $K_i^{app}$  values of 12.42  $\mu$ M and 36.62  $\mu$ M respectively. However, the mALS showed no resistance to imazethapyr and imazaquin. With regard to kinetic parameters and herbicide resistance, that A29 residue was very likely to locate at the common binding site of the sulfonylureas instead of that of imidazolinones. Several mutations leading to resistance to sulfonylureas but not to imidazolinones have been found previously. The D379 N mutant of yeast ALS was resistant only to sulfonylureas (Duggleby et al. 2003). In tobacco ALS, the D374E mutant was strongly resistant to the sulfonylurea Londax, but not the imidazolinone Carade (Le et al. 2005).

#### Discussion

Metsulfuron-methyl blocks growth of *Pseudomonas aeruginosa* by inhibition of ALS. We have reported the molecular

**Table 1** Kinetic properties of wALS and mALS

<i>P. aeruginosa</i> ALS	$K_m$	$K_c$		Specific activity (U)
	Pyruvate (mM)	Thpp ( $\mu$ M)	FAD ( $\mu$ M)	
wALS	3.14 $\pm$ 0.27	1.07 $\pm$ 0.13	0.043 $\pm$ 0.002	0.287 $\pm$ 0.021
mALS	12.54 $\pm$ 0.76	2.57 $\pm$ 0.25	0.081 $\pm$ 0.046	0.213 $\pm$ 0.012

**Table 2**  $K_i^{app}$  values of the tested herbicides by wALS and mALS

<i>P. aeruginosa</i> ALS	$K_i^{app}$			
	Metsulfuron-methyl ( $\mu$ M)	Chlorsulfuron ( $\mu$ M)	Imazethapyr (mM)	Imazaquin (mM)
wALS	0.29 $\pm$ 0.02	1.04 $\pm$ 0.23	0.93 $\pm$ 0.04	0.83 $\pm$ 0.02
mALS	12.42 $\pm$ 0.27	36.62 $\pm$ 4.31	1.03 $\pm$ 0.06	0.95 $\pm$ 0.04



basis of SM-resistance in strain L36. A mutation was found in the *ilvI* gene, which encoded the large subunit of ALS. The mutation resulted in a single amino acid substitution in the protein. The amino acid mutation was the first report of ALS-inhibitor resistant mutation in *P. aeruginosa*. The results provided new evidence to prove the substitution of valine for alanine at the corresponding amino acid residue responsible for ALS resistance to metsulfuron-methyl.

In this paper, the A29 residue is located at a common binding site for the sulfonylureas but not for the imidazolinones. This suggested that the herbicide-enzyme interactions are very complex, and that different mutations in ALS gene could affect the binding of each herbicide molecule differently, making it difficult to predict cross-resistance patterns to other ALS-inhibitors.

In this paper, the inhibition was directly measured by interaction between the purified enzyme and herbicides, without the limited diffusion of inhibitors through membrane, efflux pumps and other possible detoxification factors of bacteria (Cloete 2003). This approach has the advantage of studying the molecular mechanism of enzyme resistance to inhibitors.

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## References

- Arfin SM, Koziell DA (1973) Acetolactate synthase of *Pseudomonas aeruginosa* II. Evidence for the presence of two nonidentical subunits. *Biochim Biophys Acta* 321:356–360
- Bernasconi P, Woodworth AR, Rosen BA, Subramanian MV (1995) A naturally occurring point mutation confers broad range tolerance to herbicides that target acetolactate synthase. *J Biol Chem* 270:17381–17385
- Blair AM, Martin TD (1988) A review of the activity, fate and mode of action of sulfonylurea herbicides. *Pestic Sci* 22:195–219
- Brown HM (1990) Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. *Pestic Sci* 29:263–281
- Chang AK, Duggleby RG (1998) Herbicide-resistant forms of *Arabidopsis thaliana* acetohydroxyacid synthase: characterization of the catalytic properties and sensitivity to inhibitors of four defined mutants. *Biochem J* 333:765–777
- Chipman DM, Barak Z, Schloss JV (1998) Biosynthesis of 2-aceto-2-hydroxy acids: acetolactate synthases and acetohydroxyacid synthases. *Biochim Biophys Acta* 1385:401–419
- Chipman DM, Duggleby RG, Tittmann K (2005) Mechanisms of acetohydroxyacid synthases. *Curr Opin Chem Biol* 9:1–7
- Chong CK, Choi JD (2000) Amino acid residues conferring herbicide tolerance in tobacco acetolactate synthase. *Biochem Biophys Res Commun* 279:462–467
- Cloete TE (2003) Resistance mechanisms of bacteria to antimicrobial compounds. *Inter Biodeter Biodegr* 51:277–282
- Duggleby RG, Pang SS, Yu H, Luke W (2003) Guddat Systematic characterization of mutations in yeast acetohydroxyacid synthase. *Eur J Biochem* 270:2895–2904
- Engel S, Vyazmensky M, Vinogradov M, Berkovich V, Bar-Ilan A, Qimron U, Rosiansky Y, Barak Z, Chipman DM (2004) Role of conserved arginine in the mechanism of acetohydroxyacid synthase. *J Biol Chem* 279:24803–24812
- Ferrari A, Brusa T, Rutili A, Canzi E, Biavati B (1994) Isolation and characterization of *Methanobrevibacter oralis* sp. nov. *Curr Microbiol* 29:7–12
- Haughn GW, Somerville CR (1986) Sulfonylurea-resistant mutants of *Arabidopsis thaliana*. *Mol Gen Genet* 204:430–434
- Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) *Bergey's manual of determinative bacteriology*, 9th edn. Williams and Wilkins, Baltimore
- Ibdah M, Bar-Ilan A, Livnah O, Schloss JV, Barak Z, Chipman DM (1996) Homology modeling of the structure of bacterial acetohydroxy acid synthase and examination of the active site by site-directed mutagenesis. *Biochemistry* 35:16282–16291
- Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. Wiley, Chichester, pp 371–375
- Le DT, Yoon MY, Tae Kim Y, Choi JD (2005) Two consecutive aspartic acid residues conferring herbicide resistance in tobacco acetohydroxy acid synthase. *Biochim Biophys Acta* 1749:103–112
- Pang SS, Duggleby RG, Guddat LW (2002) Crystal structure of yeast acetohydroxyacid synthase: a target for herbicidal inhibitors. *J Mol Biol* 317:249–262
- Rajasekaran K, Grula JW, Anderson DM (1996) Selection and characterization of mutant cotton (*Gossypium hirsutum* L.) cell lines resistant to sulfonylurea and imidazolinone herbicides. *Plant Sci* 119:115–124
- Schloss JV, Dyk KEV, Vasta JF, Kutny BM (1985) Purification and properties of *Salmonella typhimurium* acetolactate synthase isozyme II from *Escherichia coli* HB101/pDU9. *Biochemistry* 24:4952–4959
- Squires CH, DeFelice M, Devereux J, Calvo JM (1983) Molecular structure of *ilvIH* and its evolutionary relationship to *ilvG* in *Escherichia coli* K12. *Nucleic Acids Res* 11:5299–5313
- Stover KC, Pham XQ, Erwin AL et al (2000) Complete genome sequence of *Pseudomonas aeruginosa* PAO1: an opportunistic pathogen. *Nature* 406:959–964
- Sutton A, Freundlich M (1980) Regulation by cyclic AMP of the *ilvB*-encoded biosynthetic acetohydroxy acid synthase in *Escherichia coli* K-12. *Mol Gen Genet* 178:179–183
- Tan MK, Medd RW (2002) Characterisation of the acetolactate synthase (ALS) gene of *Raphanus raphanistrum* L. and the molecular assay of mutations associated with herbicide resistant. *Plant Sci* 163:195–205
- Toschka HY, Hoepfl P, Ludwig W, Schleifer KH, Ulbrich N, Erdmann VA (1988) Complete nucleotide sequence of a 16S ribosomal RNA gene from *Pseudomonas aeruginosa*. *Nucl Acids Res* 16:2348
- Wei W, Zhou Y, Wang X, Huang X, Lai R (2008) *Sphingobacterium anhuiense* sp. nov., isolated from forest soil. *Int J Syst Evol Microbiol* 58:2098–2101
- Wek RC, Hauser CA, Hatfield GW (1985) The nucleotide sequence of the *ilvBN* operon of *Escherichia coli*: sequence homologies of the acetohydroxy acid synthase isozymes. *Nucl Acids Res* 13:3995–4010
- Yadav NS, McDevitt RE, Benard S, Falco SC (1986) Single amino acid substitutions in the enzyme acetolactate synthase confer resistance to the herbicide sulfometuron methyl. *Proc Natl Acad Sci USA* 83:4418–4422
- Yoon TY, Chung SM, Chang SI, Yoon MY, Hahn TR, Choia JD (2002) Roles of lysine 219 and 255 residues in tobacco acetolactate synthase. *Biochem Biophys Res Commun* 293:433–439

Zhu K, Bayles DO, Xiong A, Jayaswal RK, Wilkinson BJ (2005) Precursor and temperature modulation of fatty acid composition and growth of *Listeria monocytogenes* cold-sensitive mutants

with transposon-interrupted branched-chain alpha-keto acid dehydrogenase. *Microbiology* 151:615–623