

Insight into the Characteristics and New Mechanism of Nicosulfuron Biodegradation by a *Pseudomonas* sp. LAM1902

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ABSTRACT: A total of five strains of nicosulfuron-degrading bacteria were isolated from a continuously cultivated microbial consortium using culturomics. Among them, a novel *Pseudomonas* strain, LAM1902, with the highest degradation efficiency was investigated in detail. The characteristics of nicosulfuron-degradation by LAM1902 were investigated and optimized by response surface analysis. Furthermore, non-targeted metabolomic analysis of extracellular and intracellular biodegradation of nicosulfuron by LAM1902 was carried out by liquid chromatography/mass spectroscopy (LC–MS) and gas chromatography–time-of-flight/mass spectroscopy (GC–TOF/MS). It was found that nicosulfuron was degraded by LAM1902 mainly via breaking the sulfonylurea bridge, and this degradation might be attributed to oxalate accumulation. The results of GC–TOF/MS also showed that the intracellular degradation of nicosulfuron did not occur. However, nicosulfuron exerted a significant influence on the metabolism of inositol phosphate, pyrimidine, arginine/proline, glyoxylate, and dicarboxylate metabolism and streptomycin biosynthesis. The changes of *myo*-inositol, trehalose, and 3-aminoisobutanoic acid were proposed as a mechanism of self-protection against nicosulfuron stress.

KEYWORDS: *Pseudomonas*, nicosulfuron, microbial consortium, culturomics, response surface analysis, non-targeted metabolomics

1. INTRODUCTION

Since their discovery in the mid-1970s, sulfonylurea herbicides have been used worldwide to control weeds in many agricultural crops, such as rice paddies, maize, and wheat.¹ According to the forecast of the herbicide market based on published statistics, the sale of sulfonylurea herbicides will reach \$2.195 billion in 2019 (http://www.agroinfo.com.cn/other_detail_2369.html). Among them, nicosulfuron (2-[(4,6-dimethoxy-pyrimidin-2-yl)carbamoyl]sulfamoyl]-*N,N*-dimethylnicotinamide), which is mainly used for the control of weeds in corn fields, is the most widely used of the sulfonylurea herbicides. Although nicosulfuron is used in crops at a low agronomic dose, the molecule is frequently observed in surface and ground-waters because nicosulfuron is more persistent than other sulfonylurea herbicides like rimsulfuron and sulfosulfuron.² The long-term and widespread use of nicosulfuron has resulted in herbicide residues in the soil that may endanger food production security due to the phytotoxicity to sensitive crops.^{3,4} For example, soybeans no longer grow well in the corn fields of northeast China in recent years, possibly due to the long-term use of herbicides.⁵ In addition, several reports have shown that herbicide residues influence the activity and diversity of soil microbes and increase the resistance of weeds.⁶ Rachedi et al. confirmed that the number of *Actinobacteria* isolated from the sulfonylurea herbicide-treated agricultural soil was lower than that in the untreated soil.⁷ Moreover, it is particularly noteworthy that herbicide preparations containing metsulfuron-methyl and ethametsulfuron-methyl have been banned in China because these sulfonylurea herbicides have a long residual period and

are susceptible to phytotoxicity to the later crops. Therefore, it is considered an urgent issue to solve the phytotoxicity problems and potential risks of nicosulfuron in the environment.

Studies that investigated the fate and behavior of nicosulfuron in the natural environment showed that sulfonylurea herbicides are degraded by means of photolysis, chemical hydrolysis, and microbial degradation.^{8–10} However, photolysis of sulfonylurea herbicides only occurs on the surface of the soil, and chemical hydrolysis is limited to degradation in acidic and neutral soils, with the degradation efficiency being lower in aqueous solution.^{11,12} In contrast, microbial degradation of nicosulfuron is the primary process of removing nicosulfuron from environments, and biodegradation is the most effective and environmentally friendly mechanism. To date, nicosulfuron-degrading strains have been isolated from activated sludge, agricultural soil, and river ecosystems. For example, *Alcaligenes faecalis* ZWS11 can degrade 79.7% of 10 mg/kg nicosulfuron in 6 days,¹³ and *Bacillus subtilis* YB1, which was isolated from activated sludge, degraded 90.0% of 2 mg/kg in 5 days.^{14,15} Other bacteria, including *Bacillus megaterium* Mes11,¹⁶ *Klebsiella* sp. Y1,¹⁷ *Oceanisphaera psychrotolerans* LAM-WHM-ZC,¹⁸ *Ochrobactrum* sp. ZWS16,¹⁹ *Pseudomonas fluorescens* SG-1,²⁰ *Pseudomonas nitro-*

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