Minireview

Molecular mechanisms underlying glyphosate resistance in bacteria

Robert Hertel,1 Johannes Gibhardt,1 Marion Martienssen,2 Ramona Kuhn2 and Fabian M. Commichau
1FG Synthetic Microbiology, Institute for Biotechnology, BTU Cottbus-Senftenberg, Senftenberg, 01968, Germany.
2Institute of Environmental Technology, Chair of Biotechnology of Water Treatment, BTU Cottbus-Senftenberg, Cottbus, 03046, Germany.

Summary
Glyphosate is a nonselective herbicide that kills weeds and other plants competing with crops. Glyphosate specifically inhibits the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, thereby depleting the cell of EPSP serving as a precursor for biosynthesis of aromatic amino acids. Glyphosate is considered to be toxicologically safe for animals and humans. Therefore, it became the most-important herbicide in agriculture. However, its intensive application in agriculture is a serious environmental issue because it may negatively affect the biodiversity. A few years after the discovery of the mode of action of glyphosate, it has been observed that bacteria evolve glyphosate resistance by acquiring mutations in the EPSP synthase gene, rendering the encoded enzyme less sensitive to the herbicide. The identification of glyphosate-resistant EPSP synthase variants paved the way for engineering crops tolerating increased amounts of the herbicide. This review intends to summarize the molecular mechanisms underlying glyphosate resistance in bacteria. Bacteria can evolve glyphosate resistance by (i) reducing glyphosate sensitivity or elevating production of the EPSP synthase, by (ii) degrading or (iii) detoxifying glyphosate and by (iv) decreasing the uptake or increasing the export of the herbicide. The variety of glyphosate resistance mechanisms illustrates the adaptability of bacteria to anthropogenic substances due to genomic alterations.

Introduction
Glyphosate (N-(phosphonomethyl)glycine) is a non-selective herbicide that is used in agriculture to kill weeds (Franz, 1979; Duke and Powles, 2008). Glyphosate was first synthesized in 1950 by the Swiss chemist Henri Martin, who, however, did not discover its herbicidal effects (Franz et al., 1997; Zimdahl, 2010). Twenty years later, John E. Franz, a chemist working at the agrochemical and agricultural biotechnology corporation Monsanto, observed that glyphosate kills plants (Franz et al., 1997; Zimdahl, 2010). Glyphosate was soon patented for herbicide use and its large-scale production began in the second half of the 1970s (Grossbard and Atkinson, 1985; Duke and Powles, 2008; Benbrook, 2016). Since then, glyphosate has become the most widely used herbicide in global agriculture (Duke and Powles, 2008; Benbrook, 2016; Duke, 2018). Glyphosate is a highly polar and water-soluble compound (Franz, 1979). Therefore, in its pure form, glyphosate has little effectiveness as most plants’ hydrophobic cuticle repels it. The herbicide is usually formulated with surface-active substances, so-called surfactants, to facilitate the uptake and thus the effectiveness of glyphosate in killing plants (Knowls, 1998). Once the plant has taken up glyphosate, the herbicide is transported via the phloem to the target in various plant tissues.

Glyphosate specifically inhibits the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase of the shikimate pathway in archaea, bacteria, Apicomplexa, algae, fungi and plants (Fig. 1A; Steinrücker and Amrhein, 1980, 1984; Amrhein et al., 1983; Comai et al., 1983; Schulz et al., 1984; Kishore and Shah, 1988; Roberts et al., 1998; Roberts et al., 2002; Zhi et al., 2014). The EPSP synthase converts the glycolytic intermediate phosphoenolpyruvate (PEP) and shikimic acid-3-phosphate (S3P) into EPSP,
Fig. 1. The shikimate pathway and the molecular target of glyphosate.

A. The shikimate pathway and substances inhibiting its enzymes. 7-Deoxy-sedoheptulose (7dSH) and chlorogenic acid were shown to inhibit the dehydroquinate synthase (Brilisauer et al., 2019; Neetu et al., 2020). IMB-T130, curcumin and glyphosate inhibit the dehydroquinate dehydratase (Zhu et al., 2018), the shikimate dehydrogenase (Han et al., 2006) and the enolpyruvyl 3-phosphate (EPSP) synthase (Amrhein et al., 1980) respectively.

B. Overly of the EPSP synthase structure models from Streptococcus pneumoniae in complex with shikimate 3-phosphate (S3P) and glyphosate (GS; wheat colour; PDBid: 1RF6; Park et al., 2004) and from Mycobacterium tuberculosis in complex with S3P and phosphoenolpyruvate (PEP; light orange colour; PDBid: 2O0E). The overlay structure illustrates that GS and PEP bind at the same site in the EPSP synthase.

C. Overly of structure models of the wild type Escherichia coli EPSP synthase in complex with S3P (wheat colour; PDBid: 1G6T) and of the GS-insensitive E. coli EPSP synthase Gly96Ala mutant (light orange colour; PDBid: 1MI4; Eschenburg et al., 2002). The replacement of gly96 by Ala was shown to prevent binding of GS to the EPSP synthase.

D. Bacteria and other organisms become resistant to glyphosate (GS) by accumulating mutations in the EPSP synthase gene, by overproducing the EPSP synthase due to a promoter-up mutation or due to selective amplification of the EPSP synthase gene. [Color figure can be viewed at wileyonlinelibrary.com]

© 2021 The Authors. Environmental Microbiology published by Society for Applied Microbiology and John Wiley & Sons Ltd., Environmental Microbiology, 23, 2891–2905
which serves as an essential precursor for de novo synthesis of the aromatic amino acids, phenylalanine, tyrosine and tryptophan as well as of the vitamins folic acid and menaquinone (Wilson et al., 1998; Herrmann and Weaver, 1999). Moreover, the shikimate pathway provides precursors for secondary plant metabolism (Herrmann and Weaver, 1999). Since the shikimate pathway enzymes are essential for many organisms, the pathway is an attractive target for antibiotics (Fig. 1A). The inhibition of the EPSP synthase by glyphosate results in the depletion of the cellular levels of aromatic amino acids and thus, in plants death (Gresshoff, 1979). Glyphosate also inhibits the growth of bacteria and other organisms unless the aromatic amino acids are provided by the environment (Gresshoff, 1979; Amrhein et al., 1983; Fischer et al., 1986; Wicke et al., 2019). As the glyphosate-induced deficiency in aromatic amino acids impairs bacterial growth, it is not surprising that the herbicide has a severe effect on the general physiology of the bacteria (Kang et al., 2011; Lu et al., 2013). For instance, a transcriptome analysis of *Escherichia coli* exposed to glyphosate identified the differential expression of more than 1000 genes, representing about 23% of the genome (Lu et al., 2013). Moreover, sublethal concentrations of glyphosate reduce the susceptibility of enterobacteria to clinically important antibiotics (Kurenbach et al., 2015; Pöppe et al., 2020). Recently, it has been observed that the application of glyphosate increases the prevalence of antibiotic resistance genes in soil microbiomes (Liao et al., 2021). However, this phenomenon seems to be due to an enrichment of antibiotic resistance genes and not caused by a genome-wide glyphosate-induced increase in the mutation rate (Tincher et al., 2017; Liao et al., 2021).

Although glyphosate is considered toxicologically safe for animals and humans (Li and Long, 1988), the scientific debate about the toxicity of the weedkiller glyphosate is still ongoing (Arjó et al., 2013; Klingelhöfer et al., 2020). As described above, glyphosate is applied together with surfactants for improving the uptake of the herbicide by the plants. Many studies uncovered that the toxicity of the co-formulants is in fact much higher than that of the herbicide itself (Relyea, 2005; Mesnage et al., 2013, 2019; Mesnage and Antoniou, 2017; Defarge et al., 2018; Hao et al., 2019).

The existing scientific literature also contains conflicting results about the role of glyphosate in perturbing or changing the microbial activity and composition in the soil. For instance, it has been observed that the microbial activity and the composition of the soil, which had been exposed to glyphosate was significantly stimulated and changed respectively (Haney et al., 2002; Araújo et al., 2003). Other studies demonstrate that the treatment of cultivated soil with glyphosate only slightly altered the composition of the microbial community (Barriuso et al., 2011; Schlatter et al., 2017; Dennis et al., 2018). Moreover, glyphosate did not affect the soil microbial communities associated with crops across diverse farming systems (Kepler et al., 2020). In contrast to this, the effect of glyphosate on the microbiota of animals seems to be unambiguous (Shehata et al., 2013). A recent study revealed that the composition of the honeybee gut microbiota is altered due to the exposure of pure glyphosate and glyphosate present in a commercial herbicide formulation (Motta et al., 2018; Motta et al., 2020; Motta and Moran, 2020). Moreover, glyphosate also increases the susceptibility of bees to an infection by opportunistic pathogens like *Serratia marcescens* (Motta et al., 2018). Thus, the massive global use of glyphosate in agriculture could be the reason for the decline in the populations of bees and other insects.

A few years after the discovery of the mode of action of glyphosate in 1980 (Steinrücken and Amrhein, 1980), it was observed that bacteria evolve glyphosate resistance by acquiring mutations in the EPSP synthase gene, rendering the encoded enzyme less sensitive to the herbicide (Comai et al., 1983; Barry et al., 1992). However, these observations are not surprising because bacterial populations can reach high cell densities and subpopulations may have spontaneously accumulated beneficial mutations, providing the cells with a selective growth advantage (Gunka et al., 2013). Due to the extensive use of glyphosate in agriculture, also plants evolved mechanisms of glyphosate resistance (Baerson et al., 2002; Sammons and Gaines, 2014). This review intends to summarize the molecular mechanisms underlying glyphosate resistance in bacteria. These organisms can evolve glyphosate resistance by (i) reducing glyphosate sensitivity or elevating production of the EPSP synthase, by (ii) degrading or (iii) detoxifying glyphosate and by (iv) decreasing the uptake or increasing the export of the herbicide. The variety of glyphosate resistance mechanisms illustrates the adaptability of bacteria to anthropogenic substances due to genomic alterations.

**Evolution of glyphosate resistance by decreasing the glyphosate sensitivity or elevating the production of the EPSP synthase**

Ten years after the herbicidal effects of glyphosate were discovered, its molecular target was identified (Steinrücken and Amrhein, 1980). As described above, the glyphosate-dependent inhibition of the EPSP synthase prevents the formation of the aromatic amino acids, phenylalanine, tryptophan and tyrosine (Fig. 1A). Biochemical and structural analyses of the EPSP synthase from *E. coli* revealed that the binding of the two substrates PEP and S3P cause a large conformational
change in the enzyme resulting in a catalytically competent active site (Anton et al., 1983; Schönbrunn et al., 2001; Priestman et al., 2005; Pollegioni et al., 2011). The crystal structures of the enzyme in complex with S3P and glyphosate suggests that the herbicide acts as a transition state analogue (Fig. 1B; Schönbrunn et al., 2001; Park et al., 2004; Pollegioni et al., 2011). In fact, glyphosate replaces PEP in the active site of the EPSP synthase, thereby preventing the synthesis of EPSP, which is the precursor of de novo synthesis of the aromatic amino acids.

To enable glyphosate application on fields for killing unwanted plants competing with crops, it was necessary to develop crops containing a glyphosate-insensitive EPSP synthase. The first EPSP synthase variant carrying a single amino acid substitution (Pro101Ser) and showing a reduced sensitivity towards glyphosate was identified in the enteric bacterium *Salmonella typhimurium*. For this purpose, *S. typhimurium* was grown in the presence of toxic glyphosate levels (0.35 g L⁻¹ – 2 g L⁻¹) and subjected to chemical mutagenesis to facilitate the evolution of an *aroA* gene encoding an EPSP synthase, less sensitive to glyphosate (Comai et al., 1983; Stalker et al., 1985). The *aroA* gene was used to generate the first genetically modified tobacco plant (*Nicotiana tabacum*), tolerating increased levels of glyphosate (Comai et al., 1985). Even though the genetically modified plant was still sensitive to glyphosate, the work by Comai and colleagues (,1985), paved the way for the development of further EPSP synthase variants with reduced glyphosate sensitivity (della-Cioppa et al., 1987; Padgette et al., 1995; Liu et al., 2015; Liang et al., 2017; Fartyal et al., 2018; Liu and Cao, 2018). In the following years, insensitive EPSP synthase variants from various organisms have been obtained by directed evolution and site-directed mutagenesis as well as by cultivating the organism of choice in the presence of toxic glyphosate levels (Padgette et al., 1991; Eschenburg et al., 2002; Healy-Fried et al., 2007; Kahrizi et al., 2007; Funke et al., 2009; Cao et al., 2012; Firdous et al., 2018; Wicke et al., 2019). A glyphosate-insensitive EPSP synthase variant was also identified in the environmental *Agrobacterium* sp. strain CP4 that was isolated from the waste feed of a glyphosate production factory (Barry et al., 1992). The EPSP synthase variant from the *Agrobacterium* sp. CP4 strain was used to generate the Roundup Ready® soybean, which tolerates high amounts of glyphosate (Barry et al., 1992). Thus, glyphosate resistance by target modification in bacteria also evolves in nature. Structural analysis of the *Agrobacterium* sp. CP4 EPSP synthase revealed that an alanine residue at position 100 ( Ala100) does not allow glyphosate to bind to the active site (Funke et al., 2006). Usually, in the EPSP synthase of plants, there is a glycine residue at this position, which makes the active site accessible for glyphosate (Pollegioni et al., 2011). The same amino acid exchange in the EPSP synthase of *Klebsiella pneumoniae* and *E. coli* (Gly96Ala) also increases glyphosate resistance of the bacteria (Fig. 1C; Sost and Amrhein, 1990; Eschenburg et al., 2002). Like in bacteria, the modification of the glyphosate target by the acquisition of mutations is also a very widespread resistance mechanism in plants (Baerson et al., 2002; Heap, 2014; Sammons and Gaines, 2014; Zhang et al., 2017; Wicke et al., 2019; McElroy, 2020).

Interestingly, a recent study with the Gram-positive soil bacterium *Bacillus subtilis* revealed that the EPSP synthase does not permit any changes that increase the resistance of the enzyme to glyphosate (Wicke et al., 2019). On the one hand, any change in the amino acid sequence of the *B. subtilis* EPSP synthase could reduce the enzyme activity too much to permit the survival of the bacteria. On the other hand, the limited evolvability of the *B. subtilis* EPSP synthase could be due to the essentiality of the enzyme (Jordan et al., 2002; Koo et al., 2017; Wicke et al., 2019). Alternatively, the enzyme could fulfill additional cellular functions, which could be disturbed through any change in the amino acid sequence. It will be very interesting to elucidate the underlying reason for the essentiality of the *aroE* encoding EPSP synthase gene in *B. subtilis*.

Beside direct modification of the EPSP synthase gene, bacteria can also evolve glyphosate resistance by elevating the production of the EPSP synthase. The elevated production of the EPSP synthase can be achieved in two ways: (i) by overexpressing the coding gene or (ii), by gene amplification (Fig. 1D; Chekan et al., 2016). Indeed, *E. coli* mutants with enhanced expression of the *aroA* EPSP synthase gene due to promoter-up mutations can easily be isolated by incubating the bacteria with sublethal amounts of glyphosate (Amrhein et al., 1980; Stalker et al., 1985; Wicke et al., 2019). Moreover, the glyphosate sensitivity of bacteria can be simply reduced by overexpression the *aroA* gene using a multicopy plasmid (Rogers et al., 1983). The elevated cellular levels of the EPSP synthase reduce the toxicity of the herbicide by titrating the herbicide away, allowing a subfraction consisting of a non-inhibited enzyme to synthesize sufficient EPSP for amino acid biosynthesis (Fig. 1D). The enhanced cellular levels of the EPSP synthase due to selective gene amplification increase the resistance to glyphosate in the same way (Fig. 1D). This mechanism of glyphosate resistance can be observed in bacteria (Wicke et al., 2019) and even seems to be the dominant resistance mechanism in plants (Gaines et al., 2010, 2011; Jugulam et al., 2014; Dillon et al., 2017).

To conclude, the evolution of glyphosate resistance by overexpressing a glyphosate-sensitive EPSP synthase is
Glyphosate resistance due to degradation of the herbicide

Glyphosate belongs to the large group of phosphonic acids or amino phosphonates (Studnik et al., 2015). As other phosphonates, glyphosate is resistant to chemical hydrolysis, thermal decomposition and photolysis due to a stable C–P bond (Kononova and Nesmeyanova, 2002). Given the fact that large quantities of glyphosate have been used in agriculture, it is not surprising that microorganisms exist that can break down the herbicide (Borggaard and Gimsing, 2008; for excellent reviews see Singh and Walker, 2006; Hove-Jensen et al., 2014). Indeed, several Gram-negative and Gram-positive bacteria have been described to degrade glyphosate and/or use the herbicide as a source of phosphorous (Shinabarger et al., 1986; Pipke et al., 1987a; 1987b; Fitzgibbon and Braymer, 1988; Pipke and Amrhein, 1988a; 1988b; Liu et al., 1991; Dick and Quinn, 1995; Penaloza-Vazquez et al., 1995; Castro Jr et al., 2007; Sviridov et al., 2011, 2012, 2015; Kryuchkova et al., 2014; Yu et al., 2015). More recently, it has been demonstrated that glyphosate degradation seems to occur also in fungi and plants (see below; Duke, 2011; Rojano-Delgado et al., 2012; Vemanna et al., 2017; Pan et al., 2019). However, it is likely that the degradation pathways evolved not specifically to decompose glyphosate, but to protect the cells from naturally occurring phosphonates and to access the phosphorous present in these phosphonates or to exploit them as a phosphorous source during phosphate starvation (Metcalf and van der Donk, 2009). However, the pathways for breaking down glyphosate can also be considered as a mechanism conferring resistance to the herbicide.

So far, several glyphosate degradation pathways have been identified in microorganisms. For the details of the underlying biochemistry of the pathways, we would like to refer to the excellent review by Howe and coworkers (Hove-Jensen et al., 2014). The so-called C–P-lyase pathway is widespread among bacteria. It is the only pathway that enables the bacteria to release phosphorous from glyphosate (Fig. 2; Villarreal-Chiu et al., 2012; Hove-Jensen et al., 2014; Manav et al., 2018). Upon phosphate starvation, glyphosate is taken up via ATP binding cassette (ABC) transport systems in proteobacteria and cyanobacteria (Hove-Jensen et al., 2014). In the first step of the C–P-lyase pathway, glyphosate is converted to 5-phosphoribosyl-1-diphosphate (PRPP) and N-methylglycine (sarcosine; Zeleznick et al., 1963; Shames et al., 1987; Murata et al., 1988; Zhang and van der Donk, 2012; Seweryn et al., 2015). N-Methylglycine may be converted to glycine via the transfer of the methyl group to tetrahydrofolic acid in C1 metabolism (Hassan-Abdallah et al., 2005). Alternatively, N-methylglycine may be converted to H2O2, formaldehyde and glycine (Mesky et al., 2001). Independent of the fate of N-methylglycine in metabolism, in both cases, the inorganic phosphate derived from glyphosate may be released from PRPP (Fig. 2).

The C–P-lyase is a multi-enzyme complex that has been investigated for more than four decades in *Escherichia coli* and other bacteria (Kamat et al., 2011; Hove-Jensen et al., 2014). The enzyme complex consists of five subunits, which are involved in the transport, metabolism of the intermediates and products and in the preparation of the final cleavage via a radical reaction and post-catalytic metabolism (Kamat et al., 2011). The multi-enzyme complex is encoded by the *phnCDEFGHJKLMNOP* operon whose expression is regulated by the phosphate regulon (Villarreal-Chiu et al., 2012). The C–P-lyase cleaves a wide range of phosphonates.
including glyphosate. Interestingly, the final cleavage of the C—P bond can lead to methane release (Kamat et al., 2013).

Another pathway that is responsible for glyphosate degradation begins with the \( \mathrm{O}_2 \)-dependent oxidation reaction, which can be catalysed by a glyphosate oxidoreductase (GOX) and glycine oxidase (Fig. 2; Pollegioni et al., 2011). The oxidation of glyphosate yields in aminomethylphosphonate (AMPA) and glyoxylic acid. AMPA can be further converted by the enzymes of the C—P-lyase pathway and phosphate may be released from PRPP (Fig. 2). The glyoxylate can be converted to \( \mathrm{CO}_2 \) in the glyoxylic acid cycle. The pathway of glyphosate degradation via AMPA is less abundant in nature (Hove-Jensen et al., 2014)). However, it has been suggested that the prolonged exposure of environmental bacteria to glyphosate has led to alterations in one or more genes for improving their capacity for glyphosate cleavage (Hove-Jensen et al., 2014). Indeed, the bacterium Achromobacter sp. strain LBAA that was isolated from a glyphosate waste stream treatment facility, uses a GOX that confers glyphosate resistance to tobacco plants in a modified form (Barry and Kishore, 1995).

In recent years, other enzymes like phosphonoacetalddehyde hydrolases, phosphonooacetate hydrolases and phosphonyopruvate hydrolases that degrade phosphonates have been discovered (Huang et al., 2005; Agarwal et al., 2011; Villarreal-Chiu et al., 2012). These enzymes are also known collectively as ‘phosphonatases’, which often have a high substrate specificity. Another class of enzymes promotes the 2-oxoglutarate-dependent cleavage of phosphonates. This pathway seems to be closely associated with the \( \text{phn} \) operon encoding the C—P-lyase activity (McSorley et al., 2012).

It has also been observed that glyphosate degradation can occur by other means. For instance, the \( \text{igrA} \) (increased glyphosate-resistant gene A) gene product of the Pseudomonas sp. strain PG2982 increases glyphosate resistance of the bacterium (Fitzgibbon and Braymer, 1988, 1990). A recent study uncovered that IgrA belongs to a superfamily of \( \text{NADPH} + \mathrm{H}^+ \)-dependent aldo-keto-reductases (AKR), which are present in a variety of organisms including fungi and plants (Jez and Penning, 2001; Vemanna et al., 2017; Pan et al., 2019). IgrA (AKR1) of the Pseudomonas sp. strain PG2982 as well as other AKR homologues bind glyphosate and convert it to AMPA and glyoxylate (Pan et al., 2019). Recently, it has been demonstrated that overproduction of IgrA also increases glyphosate resistance in transgenic rice (Fartyal et al., 2018). Moreover, enzymes that do not play an obvious role in breaking down glyphosate can be modified via directed evolution in such a way that they degrade the herbicide. For instance, the \( \text{B. subtilis} \) glycine oxidase ThiO, which converts a variety of substrates (Nishiya and Imanaka, 1998; Job et al., 2002; Molla et al., 2003; Pedotti et al., 2009a; 2009b), was also subjected to directed evolution for generating enzyme variants that are useful to obtain crops with enhanced glyphosate resistance (Pedotti et al., 2009a; 2009b). The glycine oxidase is essential for thiamine biosynthesis in \( \text{B. subtilis} \) (Settembre et al., 2003). A variant of the glycine oxidase carrying three amino acid exchanges showed a 210-fold enhanced catalytic efficiency towards glyphosate (Pedotti et al., 2009a; 2009b). The expression of a plant-optimized variant of the \( \text{B. subtilis} \) glycine oxidase indeed conferred glyphosate resistance to alfalfa (Medicago sp.; Nicotia et al., 2014). To conclude, native promiscuous enzymes of bacteria, fungi and plants can be employed to reduce the toxicity of glyphosate (Duke, 2011; Rojano-Delgado et al., 2012; Vemanna et al., 2017; Pan et al., 2019).

**Glyphosate detoxification by covalent modification**

Glyphosate resistance might also occur by other means (Pollegioni et al., 2011). It has been observed that Streptomyces and other species producing glufosinate (phosphonotricin), which inhibits the glutamine synthetase (Bayer et al., 1972; Fraser and Ridley, 1984; VanDriss et al., 2016), protect themselves by converting the harmful substance to a non-inhibitory acetylated form (Fig. 3A; Whermann et al., 1996). The detoxification of harmful substances by covalent modification is a very common strategy among antibiotic producers (i.e., Streptomyces species). Similar to the \( N \)-acylated glufosinate, it has been demonstrated that also the \( N \)-acylated form of glyphosate does not inhibit the EPSP synthase (Fig. 3B; Castle et al., 2004). The idea that \( N \)-acylation of glyphosate provides an alternative strategy to engineer glyphosate-resistant crops stimulated the search for a glyphosate-\( N \)-acyltransferase (GAT) with high catalytic efficiency. For this purpose, several hundred Bacillus isolates were screened for their ability to generate \( N \)-acylgllyphosate (Castle et al., 2004). The screen revealed that the acyltransferase from Bacillus licheniformis showed the highest GAT activity. After identifying the \( \text{gat} \) gene, the GAT was subjected to directed evolution to improve the catalytic efficiency of the enzyme, yielding a variety of variants (Castle et al., 2004; Siehl et al., 2005, 2007). One of the GAT variants was structurally analysed to get insights into the reaction mechanism of the enzyme (Siehl et al., 2007; Pollegioni et al., 2011). Recently, glyphosate acetylation was reported to occur in Achromobacter sp. Kg 16 (Shushkova et al., 2016). Even though the natural substrate of the GAT homologues is unknown, the work performed on the enzyme illustrates the power of directed
The resistance to glyphosate can also be enhanced by other enzymes that covalently modify the herbicide. For instance, the hygromycin phosphotransferases Hph and GlpA from *E. coli* and *Burkholderia pseudomallei* respectively, can phosphorylate glyphosate, thereby conferring glyphosate resistance (Fig. 3C; Rao et al., 1983; Penaloza-Vazquez et al., 1995). To conclude, various enzymes have been described that confer glyphosate resistance by covalently modifying the herbicide. Since environmental bacteria often come into contact with glyphosate, certainly other enzymes will be discovered that have gained the ability to detoxify the herbicide.

**Glyphosate resistance due to decreased uptake and increased export**

Although glyphosate has been used in agriculture for almost 50 years, it has long been unknown how the herbicide enters a living cell. Recently, it has been observed that the difference in the resistance to glyphosate in different strains of baker’s yeast *Saccharomyces cerevisiae* is due to genetic variations in the genes encoding the transporters Dip5 and Pdr5 (Fig. 4; Rong-Mullins et al., 2017). Dip5 is a transporter with low substrate specificity that transports glutamate, aspartate, glutamine, asparagine, serine, alanine and glycine (Regenberg et al., 1998, 1999). Dip5 belongs to the amino acid-polyamine-organocation (APC) superfamily of transport proteins (Saier, 2000a). Pdr5 is an ABC efflux transporter that is involved in the detoxification of the yeast cell during exponential growth phase by exporting a variety of substrates (Rogers et al., 2001; Mamnun et al., 2004; Golin et al., 2007). The inactivation of the Dip5 gene increased glyphosate resistance. By contrast, the deletion of the Pdr5 encoding gene decreased glyphosate resistance. These observations suggest that Dip5 and Pdr5 are indeed involved in the uptake and export respectively, of glyphosate in yeast (Rogers et al., 2001). The study by Rong-Mullins and colleagues (2017) provides initial evidence for the involvement of amino acid transporters in translocating glyphosate across the membranes in a unicellular eukaryotic organism. It has also been reported that amino acid transporters could be involved in the transport of glyphosate across mammalian epithelial tissues (Xu et al., 2016). Recently, it has been demonstrated that the enhanced export of glyphosate via fungal and bacterial transport proteins reduces the cellular toxicity of the herbicide. For instance, the overproduction of the uncharacterized membrane proteins MFS40 and YhhS from *Aspergillus oryzae* and *E. coli* respectively, share similarity with the major facilitator secondary transporter superfamily, enhances glyphosate resistance of *E. coli* (Fig. 4; Staub et al., 2012; Tao et al., 2017). However, the precise functions of the enzymes in detoxifying harmful substances produced by the own cell or other bacteria. The GAT encoded by the gat gene, which was subjected to directed evolution, did not show evidence of allergenicity or toxicity when orally applied to mice (Delaney et al., 2008). The GAT could therefore replace glyphosate-insensitive EPSP synthase variants or both, the GAT and insensitive EPSP synthase variants, could be used to enhance the glyphosate resistance of crops (Castle et al., 2004; Guo et al., 2015; Stokstad, 2004). In the past years, the GAT has indeed been successfully introduced into tobacco and cotton to enhance their resistance to glyphosate (Liu et al., 2015; Liang et al., 2017). Furthermore, the gat gene has been used as a selection marker to genetically engineer bacteria (Norris et al., 2009).
Glufosinate resistance mediated by reduced uptake and export. GltT and GltP, Bacillus subtilis glutamate transporters; YhhS and MFS40, major facilitator secondary transporters from Escherichia coli and Aspergillus oryzae respectively; Pdr5 and Dip5 are amino acid and ABC efflux transporters respectively, from Saccharomyces cerevisiae. Glufosinate and glyphosate inhibit the glutamine synthetase (GlnA) and the EPSP synthase AnoE respectively. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 4. Glyphosate resistance mediated by reduced uptake and export. GltT and GltP, Bacillus subtilis glutamate transporters; YhhS and MFS40, major facilitator secondary transporters from Escherichia coli and Aspergillus oryzae respectively; Pdr5 and Dip5 are amino acid and ABC efflux transporters respectively, from Saccharomyces cerevisiae. Glufosinate and glyphosate inhibit the glutamine synthetase (GlnA) and the EPSP synthase AnoE respectively. [Color figure can be viewed at wileyonlinelibrary.com]

transporters remain to be determined. Experimental evidence suggests that YhhS is involved in the efflux of pentose sugars (Kolta and Rao, 2012).

Altered glyphosate transport has also been recognized as a mechanism of herbicide resistance in plants (Ge et al., 2010; Chekan et al., 2016). It has been observed that in glyphosate-resistant horseweed Conyza canadensis, the herbicide was rapidly translocated from the cytoplasm to the vacuoles (Ge et al., 2010). The molecular basis for the transport of glyphosate has been attributed to ABC transporters and a tonoplast intrinsic protein (TIP; Peng et al., 2010; Yuan et al., 2010; Nol et al., 2012). It has been suggested that TIPs regulate water transport across the membrane of the vacuole (Chrispeels and Maurel, 1994; Maurel, 1997). Currently, it is hypothesized that the TIP facilitates water influx into the cell to reduce the cellular concentration of the herbicide and that the ABC transporters translocate the herbicide into the vacuole in glyphosate-resistant horseweed (Nol et al., 2012). Thus, like in yeast, glyphosate resistance can also be mediated by altered transport of the herbicide in plants. However, the molecular details how the transport proteins mediate glyphosate resistance in plants remain to be elucidated.

A more comprehensive study about the proteins mediating glyphosate transport in bacteria was recently performed with B. subtilis (Wicke et al., 2019). Previously, it has been reported that the addition of glutamate, glutamine, proline and arginine decreases the glyphosate sensitivity of B. subtilis (Fischer et al., 1986). The amino acids probably inhibit the glyphosate uptake, thereby preventing an increase of the cellular concentration to toxic levels. In the same study, it was mentioned that the growth rate of B. subtilis in medium containing toxic amounts of glyphosate (2.5 mM), rapidly increases after prolonged incubation of the bacterial cultures, which could be due to the emergence of glyphosate-resistant B. subtilis mutants (Fischer et al., 1986). Indeed, the study by Wicke and co-workers (Wicke et al., 2019) revealed that B. subtilis rapidly evolves glyphosate resistance by acquiring loss-of-function mutations in the gltT gene encoding the high-affinity glutamate/asparate symporter GltT (Fig. 4; Zaprasis et al., 2015). Moreover, further adaptation of a gltT mutant led to the inactivation of the gltP gene encoding the low-affinity glutamate transporter GltP (Fig. 4; Tolner et al., 1995; Wicke et al., 2019). Additional genetic as well as metabolome analyses confirmed that GltT is the major glyphosate transporter in B. subtilis (Wicke et al., 2019). Furthermore, the glutamate transporter GltP of E. coli, which shares 52% overall sequence identity with GltT from B. subtilis, was also shown to mediate glyphosate transport. Both GltP and GltT belong to the dicarboxylate/amino acid:cation (Na\(^+\) or H\(^+\)) symporter family (Slotboom et al., 1999; Saier, 2000b). It is interesting to note that the suppressor screen by Wicke and co-workers (Wicke et al., 2019) did not led to the inactivation of the aimA gene that was recently shown to encode the major glutamate transporter AimA in B. subtilis (Krüger et al., 2021). It is tempting to speculate that AimA has a higher specificity for transporting glutamate as compared with GltT and GltP. Indeed, GltT also mediates transport of the herbicide glufosinate, which inhibits the glutamine synthetase (Fig. 4; Bayer et al., 1972; Fraser and Ridley, 1984; Wicke et al., 2019). To conclude, in prokaryotic as well as in eukaryotic systems glyphosate and structurally similar substances may enter the cell and organelles via amino acid transporters (Cherepenko and Karpenko, 1999; Wicke et al., 2019). In fact, it is very common among bacteria to inactivate transporter genes upon exposure to toxic amino acid analogues (Zaprasis et al., 2014; Commichau et al., 2015).

Conclusions and outlook

The variety of glyphosate resistance mechanisms that have been identified and characterized illustrate the ability of bacteria to adapt to anthropogenic substances due to genomic alterations. The characterization of glyphosate-resistant bacteria and the understanding of the molecular mechanism underlying glyphosate resistance paved the way for genetically engineering plants.
tolerating increased levels of the herbicide. These studies certainly contributed to the fact that glyphosate has become the most widely used herbicide in global agriculture. However, intensive application of glyphosate in agriculture is a serious environmental issue because it may negatively affect the biodiversity in the soil and in the insect gut microbiota. In addition to glyphosate, other substances have been identified and characterized in recent years that selectively inhibit the shikimate pathway (Fig. 1A; Han et al., 2006; Zhu et al., 2018; Brilisauer et al., 2019; Neetu et al. 2020). A very promising substance is the deoxy sugar 7-deoxy-sedoheptulose (7dSH) that is produced by the cyanobacterium Synechococcus elongatus and inhibits the dehydroquinate synthase (Fig. 1A; Brilisauer et al., 2019). It could be shown that 7dSH inhibits the growth of Arabidopsis thaliana seedlings more strongly than glyphosate when applied at the same substrate concentration (Brilisauer et al., 2019). However, even if 7dSH could be a potential substitute for glyphosate, the deoxy sugar has not yet been classified with regard to its effects on soil microbiota and other organisms. Moreover, in the future it will be interesting to evaluate whether a glyphosate-resistant B. subtilis gltT mutant lacking the GltT glyphosate transporter is suitable to identify glyphosate uptake systems from plants (Wicke et al., 2019). To address this issue, plant-derived cDNA libraries could be used to screen for B. subtilis gltT transfectants that are unable to grow with glyphosate due to restored uptake of the herbicide.

Acknowledgements

This work was supported by the Göttingen Center for Molecular Biosciences (GZMB), the Georg-August University of Göttingen and the Brandenburg Technische Universität Cottbus-Senftenberg. We are grateful to Janek Meißner for the help with some experiments. We thank Jörg Stülke and all members of the iGEM Team Göttingen 2018 for helpful discussions.

References

Agarwal, V., Borisova, S.A., Metcalf, W.W., van der Donk, W.A., and Nair, S.K. (2011) Structural and mechanistic insights into C-P bond hydrolysis by phosphonoacetate hydrolase. Chem Biol 18: 1230–1240.

Amrhein, N., Johanning, D., Schab, J., and Schulz, A. (1983) Biochemical basis for glyphosate-resistance in a bacterium and a plant tissue culture. FEBS Lett 157: 191–196.

Amrhein, N., Schab, J., and Steinrücken, H.C. (1980) The mode of action of the herbicide glyphosate. Naturwissenschaften 67: 356–357.

Anton, D.L., Hedstrom, L., Fish, S.M., and Abeles, R.H. (1983) Mechanism of enolpyruvyl shikimate-3-phosphate synthase exchange of phosphoenolpyruvate with solvent proteins. Biochemistry 22: 5903–5908.

Araújo, A.S.F., Monteiro, R.T.R., and Abarkeli, R.B. (2003) Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52: 799–804.

Arjó, G., Portero, M., Pinod, C., Vinas, J., Matias-Guiu, X., Capell, T., et al. (2013) Plurality of opinion, scientific discourse and pseudoscience: an in depth analysis of the Séralini et al., study claiming that Roundup™ ready com or the herbicide Roundup™ cause cancer in rats. Transgenic Res 22: 255–267.

Baerson, S.R., Rodríguez, D.J., Tran, M., Feng, Y., Biest, N. A., and Dill, G.M. (2002) Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylishikimate-3-phosphate synthase. Plant Physiol 129: 1265–1275.

Barriuso, J., Marin, S., and Mellado, R.P. (2011) Potential accumulative effect of the herbicide glyphosate on glyphosate-resistant maize rhizobacterial communities over a three-year cultivation period. PLoS ONE 6: e27558.

Barry, G.F. and Kishore, G.M. 1995. Glyphosate resistant plants. US Patent 5, 463,175.

Barry, G.F., Kishore, G.M., and Padgette, S.R. (1992). Int Pat., WO 92/04449.

Bayer, E., Gugel, K.H., Hagele, K., Hagemeier, H., Jessipow, S., Konig, W.A., and Zahnner, H. (1972) Stoffwechselprodukte von Mikroorganismen. 98. Mitteilung: Phosphinotricin und Phosphinotricyl-Alanyl-Alanin. Helv Chim Acta 55: 224–239.

Benbrook, C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. Environ Sci Eur 28: 3.

Borggaard, O.K., and Gimsing, A.L. (2008) Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Manag Sci 64: 441–456.

Brilisauer, K., Rapp, J., Rath, P., Schöllhorn, A., Bleul, L., Weiß, E., et al. (2019) Cyanobacterial antimetabolite 7-deoxy-sedoheptulose blocks the shikimate pathway to inhibit growth of prototrophic organisms. Nat Commun 10: 545.

Cao, G., Liu, Y., Zhang, S., Yang, X., Chen, R., Zhang, Y., et al. (2012) A novel 5-enolpyruvylishikimate-3-phosphate synthase shows high glyphosate resistance in Escherichia coli and tobacco plants. PLoS One 7: e38718.

Castle, L.A., Siehl, D.L., Gorton, R., Patten, P.A., Chen, Y. H., Bertain, S., et al. (2004) Discovery and directed evolution of a glyphosate resistance gene. Science 304: 1151–1154.

Castro, J.V., Jr., Peralba, M.C., and Ayub, M.A. (2007) Biodegradation of the herbicide glyphosate by filamentous fungi in platform shaker and batch bioreactor. J Environ Sci Health B 42: 883–886.

Chekan, J.R., Cogan, D.P., and Nair, S.K. (2016) Molecular basis for resistance against phosphonate antibiotics and herbicides. MedChemComm 7: 28–36.

Cherepenko, E., and Karpenko, O. (1999) Uptake of the herbicidal glyphosate by Escherichia coli K-12. Biosci Rep 19: 43–49.

Chrispeels, M.J., and Maurel, C. (1994) Aquaporins: the molecular basis of facilitated water movement through living plant cells? Plant Physiol 105: 9–13.

Comai, L., Facchini, D., Hiatt, W.R., Thompson, G., Rose, R. E., and Stalker, D.M. (1985) Expression in plants of a mutant arx1 gene from Salmonella typhimurium confers resistance to glyphosate. Nature 317: 741–744.
Comai, L., Sen, L.C., and Stalker, D.M. (1983) An altered aroA gene product confers resistance to the herbicide glyphosate. Science 221: 370–371.

Commichau, F.M., Alzinger, A., Sande, R., Bretzel, W., Reuß, D.R., Dormeyer, M., et al. (2015) Engineering Bacillus subtilis for the conversion of the antimetabolite 4-hydroxy-L-threonine to pyridoxine. Metab Eng 29: 196–207.

Defarge, N., Spiroix de Vendomois, J., and Séralini, G.E. (2018) Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides. Toxicol Rep 5: 156–163.

Delaney, B., Zhang, J., Carlson, G., Schmidt, J., Stagg, B., Comstock, B., et al. (2008) A gene-shuffled glyphosate acetyltransferase protein from Bacillus licheniformis (GAT4601) shows no evidence of allergenicity or toxicity. Toxicol Sci 102: 425–432.

Della-Cioppa, G., Bauer, S.C., Taylor, M.L., Rochester, D.E., Dick, R.E., and Quinn, J.P. (1995) Glyphosate-degrading Bacillus subtilis. Appl Environ Microbiol 61: 579–584.

Dennis, P.G., Kukulies, T., Forstner, C., Orton, T.G., and Pattison, A.B. (2018) The effects of glyphosate, glufosinate, pararquat and pararquat-diquat on soil microbial activity and bacterial, archaeal and nematode diversity. Sci Rep 8: 2119.

Dick, R.E., and Quinn, J.P. (1995) Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. Appl Microbiol Biotechnol 43: 545–550.

Dillon, A., Varanasi, V.K., Danilova, T.V., Koo, D.H., Nakka, S., Peterson, D.E., et al. (2017) Physical mapping of amplified copies of the 5-enolpyruvylshikimate-3-phosphate synthase gene in glyphosate-resistant Amanthus tuberculatus. Plant Physiol 173: 1226–1234.

Duke, S.O. (2011) Glyphosate degradation in glyphosate-resistant and -susceptible crops and weeds. J Agric Food Chem 59: 5835–5841.

Duke, S.O. (2018) The history and current status of glyphosate. Pest Manag Sci 74: 1027–1034.

Duke, S.O., and Powles, S.B. (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64: 319–325.

Eschenburg, S., Healy, M.L., Priestman, M.A., Lushington, G.H., and Schönbrunn, E. (2002) How the mutation glycine96 to alanine confers glyphosate insensitivity to 5-enolpyruvyl shikimate-3-phosphate synthase from Escherichia coli. Planta 216: 129–135.

Fartyal, D., Agarwal, A., James, D., Borphukan, B., Ram, B., Sheri, V., et al. (2018) Co-expression of P1735 mutant rice EPSPS and igA genes results in higher glyphosate resistance in transgenic rice. Front Plant Sci 13: 144.

Firdous, S., Iqbal, S., Anwar, S., and Jabeen, H. (2018) Identification and analysis of 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) gene from glyphosate-resistant Ochrobactrum intermedium Ssq20. Pest Manag Sci 74: 1184–1196.

Fischer, R.S., Berry, A., Gaines, C.G., and Jensen, R.A. (1986) Comparative action of glyphosate as a trigger of energy drain in eubacteria. J Bacteriol 168: 1147–1154.

Fitzgibbon, J., and Braymer, H.D. (1988) Phosphate starvation induces uptake of glyphosate by Pseudomonas sp. strain PG2982. Appl Environ Microbiol 54: 1886–1888.

Fitzgibbon, J., and Braymer, H.D. (1990) Cloning of a gene from Pseudomonas sp. strain PG2982 conferring increased glyphosate resistance. Appl Environ Microbiol 56: 3382–3388.

Franz, J.E. (1979). In Advances in Pesticide Science, Vol. 2, Geissbuehler, H. (ed.). Oxford and New York: Pergamon Press, pp. 139–147.

Franz, J.E., Mao, M.K., and Sikorski, J.A. (1997). Glyphosate: a unique and global herbicide. ACS monograph No. 189. American Chemical Society, Washington, DC, 653 pp.

Fraser, A.R., and Ridley, S.M. (1984) Kinetics for glutamine-synthetase inhibition by phosphinothricin and measurement of other enzyme activities in situ in isolated asparagus cells using freeze-thaw technique. Planta 161: 470–474.

Funke, T., Han, H., Healy-Fried, M.L., Fischer, M., and Schönbrunn, E. (2006) Molecular basis for the herbicide resistance of Roundup Ready crops. Proc Natl Acad Sci U S A 103: 13010–13015.

Funke, T., Yang, Y., Han, H., Healy-Fried, M., Olesen, S., Becker, A., Schönbrunn, E. 2009. Structural basis of glyphosate resistance resulting from the double mutation Thr39 → Ile and Pro101 → Ser in 5-enolpyruvylshikimate-3-phosphate synthase from Escherichia coli. J Biol Chem 284: 9854–9860.

Gaines, T.A., Shaner, D.L., Ward, S.M., Leach, J.E., Preston, C., and Westra, P. (2011) Mechanism of resistance of evolved glyphosate-resistant Palmer amaranth (Amaranthus palmeri). J Agric Food Chem 59: 5886–5889.

Gaines, T.A., Zhang, W., Wang, D., Bukun, B., Chisholm, S. T., Shaner, D.L., et al. (2010) Gene amplification confers glyphosate resistance in Amaranthus palmeri. Proc Natl Acad Sci U S A 107: 1029–1034.

Ge, X., André d’Avignon, D., Ackerman, J.J.H., and Sammons, R.D. (2010) Rapid vacuolar sequestration: the horseradish glyphosate resistance mechanism. Pest Manag Sci 66: 345–348.

Golin, J., Ambudkar, S.V., and May, L. (2007) The yeast Pdr5p multidrug transporter: how does it recognize so many substrates? Biochim Biophys Res Commun 356: 1–5.

Gresshoff, P.M. (1979) Growth inhibition by glyphosate and reversal of its actions by phenylalanine and tyrosine. Aust J Plant Physiol 6: 177–185.

Grossbard, E., and Atkinson, D. (eds). (1985) The Herbicide Glyphosate. London, UK: Butterworth, p. 490.

Gunka, K., Stannek, L., Care, R.A., and Commichau, F.M. (2013) Selection-driven accumulation of suppressor mutants in Bacillus subtilis: the apparent high mutation frequency of the cryptic gudB gene and the rapid clonal expansion of gudB+ suppressors are due to growth under selection. F1000Res One 8: e66120.

Guo, B., Guo, Y., Hong, H., Jin, L., Zhang, L., Chang, R. Z., et al. (2015) Co-expression of G2-EPSPS and glyphosate acetyltransferase GAT genes conferring high resistance to glyphosate in soybean. Front Plant Sci 15: 847.
Haney, R.L., Senseman, S.A., and Hons, F.M. (2002) Biochemical characterization and inhibitor discovery of shikimate dehydrogenase from Helicobacter pylori. FEBS J 273: 4682–4692.

Haney, R.L., Senseman, S.A., and Hons, F.M. (2002) Effect of roundup ultra on microbial activity and biomass from selected soils. J Environ Qual 31: 730–735.

Hao, Y., Zhang, Y., Ni, H., Gao, J., Yang, J., Xu, W., and Tao, L. (2019) Evaluation of the cytotoxic effects of glyphosate herbicides in human liver, lung, and nerve. J Environ Sci Health B 54: 737–744.

Hassan-Abdallah, A., Zhao, G., Eschenbrenner, M., Chen, Z.W., Mathews, F.S., and Jorns, M.S. (2005) Cloning, expression and crystallization of heterotetrameric sarcosine oxidase from Pseudomonas maltophilia. Protein Expr Purif 43: 33–43.

Healy-Fried, M.L., Funke, T., Priestman, M.A., Han, H., and Schönbrunn, E. (2007) Structural basis of glyphosate resistance resulting from mutations of PrO101 in Escherichia coli 5 enolpyruvylshikimate-3-phosphate synthase. J Biol Chem 282: 32949–32955.

Heap, I. (2014) Global perspective of herbicide-resistant weeds. Pest Manag Sci 70: 1306–1315.

Herrmann, K.M., and Weaver, L.M. (1999) The shikimate pathway. Annu Rev Plant Physiol Plant Mol Biol 50: 473–503.

Hove-Jensen, B., Zechel, D.L., and Jochimsen, B. (2014) Utilization of glyphosate as phosphate source: biochemistry and genetics of bacterial carbon-phosphorus lyase. Microbiol Mol Biol Rev 78: 176–197.

Huang, J., Su, Z., and Xu, Y. (2005) The evolution of microbial phosphate degradative pathways. J Mol Evol 61: 682–690.

Jez, J.M., and Penning, T.M. (2001) The aldo-keto reductase (AKR) superfamily: an update. Chem Biol Interact 130-132: 499–525.

Job, V., Marcone, G.L., Pilone, M.S., and Pollegioni, L. (2002) Glycine oxidase from Bacillus subtilis. Characterization of a new flavoprotein. J Biol Chem 277: 6985–6993.

Jordan, K.I., Rogozin, I.B., Wolf, Y.I., and Koonin, E.V. (2002) Essential genes are more evolutionary conserved than are nonessential genes in bacteria. Genome Res 12: 962–968.

Jugulam, M., Niehues, K., Godar, A.S., Koo, D.H., Danilova, T., Friebel, B., et al. (2014) Tandem amplification of a chromosomal segment harboring 5-enolpyruvylshikimate-3-phosphate synthase locus confers glyphosate resistance in Kochia scoparia. Plant Physiol 166: 1200–1207.

Kahrizi, D., Salmanian, A.H., Afshari, A., Moleni, A., and Mousavi, A. (2007) Simultaneous substitution of Gly96 to Ala and Ala183 to Thr in 5-enolpyruvylshikimate-3-phosphate synthase gene of E. coli (k12) and transformation of rapseseed (Brassica napus L.) in order to make resistance to glyphosate. Plant Cell Rep 26: 95–104.

Kamat, S.S., Williams, H.J., Dangott, L.J., Chakraborti, M., and Raushel, F.M. (2013) The catalytic mechanism for aerobic formation of methane by bacteria. Nature 497: 132–136.

Kamat, S.S., Williams, H.J., and Raushel, F.M. (2011) Intermediates in the transformation of phosphonates to phosphate by bacteria. Nature 460: 570–573.

Kang, Y., Norris, M.H., Zarzycki-Siek, J., Nierman, W.C., Donachie, S.P., and Hoang, T.T. (2011) Transcript analysis from single bacterium for transcriptome analysis. Genome Res 21: 925–935.

Kantor, R.M., Schmidt, D.J.E., Yarwood, S.A., Cavigelli, M.A., Reddy, K.N., Duke, S.O., et al. (2020) Soil microbial communities in diverse agroecosystems exposed to the herbicide glyphosate. Appl Environ Microbiol 86: e01719–e01719.

Kishore, G.M., and Shah, D.M. (1988) Amino acid biosynthesis inhibitors as herbicides. Annu Rev Biochem 57: 627–663.

Klingelhöfer, D., Braun, M., Brüggmann, D., and Gronberg, D.A. (2020) Glyphosate: how do ongoing controversies, market characteristics, and funding influence the global research landscape? Sci Total Environ 765: 144271.

Knowl, D.A. (1998) Chemistry and technology of agricultural formulations. In Industrial Chemistry and Chemical Engineering. Netherlands: Springer. https://doi.org/10.1007/978-94-011-4956-3.

Kolta, K., and Rao, C.V. (2012) Identification and analysis of the putative pentose sugar efflux transporters in Escherichia coli. PLoS One 7: e43700.

Kononova, S.V., and Nesmeyanova, M.A. (2002) Phosphonates and their degradation by microorganisms. Biochemistry (Mosc) 67: 184–195.

Koo, B.M., Kritikos, G., Farelli, J.D., Todor, H., Tong, K., Kimsey, H., et al. (2017) Construction and analysis of two genome-scale deletion libraries for Bacillus subtilis. Cell Systems 4: 291–305.

Krüger, L., Herberg, C., Rath, H., Pedreira, T., Ischebeck, T., Poehein, A., et al. (2021) Essentiality of c-di-AMP in Bacillus subtilis: bypassing mutations converging in potassium and glutamate homeostasis. PLoS Genet 17: e1009092.

Kryuchkova, Y.V., Burygin, G.L., Gogoleva, N.E., Gogolev, Y.V., Chernyshova, M.P., Makarov, O.E., et al. (2014) Isolation and characterization of a glyphosate-degrading rhizosphere strain Enterobacter cloacae K7. Microbiol Res 169: 99–105.

Kurenbach, B., Marjoshi, D., Amábile-Cuevas, C.F., Ferguson, G.S., Godsoe, W., Gibson, P., and Heinemann, J. A. (2015) Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium. MBio 6: e00009-15.

Li, A.P., and Long, T.J. (1998) An evaluation of the genotoxic potential of glyphosate. Fundam Appl Toxicol 10: 537–546.

Liang, C., Sun, B., Meng, Z., Meng, Z., Wang, Y., Sun, G., et al. (2017) Co-expression of GR79 EPSPS and GAT yields herbicide-resistant cotton with low glyphosate residues. Plant Biotechnol J 15: 1622–1629.

Liao, H., Li, X., Yang, Q., Bai, Y., Cui, P., Wen, C., et al. (2021) Herbicide selection promotes antibiotic resistance in soil microbiomes. Mol Biol Evol. https://doi.org/10.1093/molbev/msab029.

Liu, C.M., McLean, P.A., Sookdeo, C.C., and Cannon, F.C. (1991) Degradation of the herbicide glyphosate by...
members of the family *Rhizobaceae*. *Appl Environ Microbiol* **57**: 1799–1804.

Liu, F., and Cao, Y. (2018) Expression of the 5-enolpyruvylshikimate-3-phosphate synthase domain from *Acremonium sp. aroM* complex enhances resistance to glyphosate. *Biotechnol Lett* **40**: 855–864.

Liu, Y., Cao, G., Chen, R., Zhang, S., Ren, Y., Lu, W., et al. (2015) Transgenic tobacco simultaneously overexpressing glyphosate-N-acetyltransferase and 5-enolpyruvylshikimate-3-phosphate synthase are more resistant to glyphosphate than those containing one gene. *Transgenic Res* **24**: 753–763.

Lu, W., Li, L., Chen, M., Zhou, Z., Zhang, W., Ping, S., et al. (2013) Genome-wide transcriptional responses of *Escherichia coli* to glyphosate, a potent inhibitor of the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phosphate synthase. *Mol Biosyst* **9**: 522–530.

Mannun, Y.M., Schüller, C., and Kuchler, K. (2004) Expression regulation of the yeast PDR5 ATP-binding cassette (ABC) transporter suggests a role in cellular detoxification during exponential growth phase. *FEBS Lett* **559**: 111–117.

Manav, M.C., Sofos, N., Hove-Jensen, B., and Brodersen, D.E. (2018) The Abc of phosphonate breakdown: a mechanism for bacterial survival. *Bioessays* **40**: e1800091.

Maurel, C. (1997) Aquaporins and water permeability of plant membranes. *Annu Rev Plant Physiol Plant Mol Biol* **48**: 399–429.

McElroy, J.S. (2020) *Echinocloa colona* with reported resistance to glyphosate conferred by aldo-keto reductase also contains a Pro-106-Thr EPSPS target site mutation. *Plant Physiol* **183**: 447–450.

McSorley, F.R., Wyatt, P.B., Martinez, A., DeLong, E.F., Hove-Jensen, B., and Zechel, D.L. (2012) PhrY and PhrZ comprise a new oxidative pathway for enzymatic cleavage of a carbon-phosphorus bond. *J Am Chem Soc* **134**: 8364–8367.

Meskys, R., Harris, R.J., Casaite, V., Basran, J., and Scrrton, N.S. (2001) Organisation of the genes involved in dimethyglycine and sarcosine degradation in *Arthrobacter* spp.: implications for glycin betaine catabolism. *Eur J Biochem* **268**: 3390–3398.

Mesnage, R., and Antoniou, M.N. (2017) Facts and fallacies in the debate on glyphosate toxicity. *Front Public Health* **5**: 316.

Mesnage, R., Benbrook, C., and Antoniou, M.N. (2019) Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. *Food Chem Toxicol* **128**: 137–145.

Mesnage, R., Bernay, B., and Séràlini, G.E. (2013) Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology* **313**: 122–128.

Metcalfe, W.W., and van der Donk, W.A. (2009) Biosynthesis of phosphonic and phosphinic acid natural products. *Annu Rev Biochem* **78**: 65–94.

Molla, G., Motteran, L., Job, V., Pilone, M.S., and Pollegioni, L. (2003) Kinetic mechanisms of glycine oxidase from *Bacillus subtilis*. *Eur J Biochem* **270**: 1474–1482.

Motta, E.V.S., Mak, M., De Jong, T.K., Powell, J.E., O’Donnell, A., Suhr, K.J., et al. (2020) Oral or topical exposure to glyphosate in herbicide formulation impacts the gut microbiota and survival rates of honey bees. *Appl Environ Microbiol* **86**: e01150–e01120.

Motta, E.V.S., and Moran, N.A. (2020) Impact of glyphosate on the honey bee gut microbiota: effects of intensity, duration, and timing of exposure. *msSystems* **5**: e00268-20.

Motta, E.V.S., Raymann, K., and Moran, N.A. (2018) Glyphosate perturbs the gut microbiota of honey bees. *Proc Natl Acad Sci U S A* **115**: 10305–10310.

Murata, K., Higaki, N., and Kimura, A. (1988) Detection of carbon-phosphorus lyase activity in cell free extracts of *Enterobacter aerogenes*. *Biochem Biophys Res Comm* **157**: 190–195.

Neetu, N., Katiki, M., Dev, A., Gaur, S., Tomar, S., and Kumar, P. (2020) Structural and biochemical analysis reveal chlorogenic acid inhibits the shikimate pathway. *J Bacteriol* **202**: e00248–e00220.

Nicolia, A., Ferradini, N., Molla, G., Biagetti, E., Pollegioni, L., Veronesi, F., and Rosellini, D. (2014) Expression of an evolved engineered variant of a bacterial glycine oxidase leads to glyphosate resistance in alfalfa. *J Biotechnol* **184**: 201–208.

Nishiya, Y., and Imanaka, T. (1998) Purification and characterization of novel glycine oxidase from *Bacillus subtilis*. *FEBS Lett* **438**: 263–266.

Nol, N., Tsikou, D., Eid, M., Livieratos, I.C., and Giannopolitis, C.N. (2012) Shikimate leaf disc assay for early detection of glyphosate resistance in *Coryza canadensis* and relative transcript levels of EPSPS and ABS transporter genes. *Weed Res* **52**: 233–241.

Norris, M.H., Kang, Y., Lu, D., Wilcox, B.A., and Hoang, T.T. (2009) Glyphosate resistance as a novel select-agent-compliant, non-antibiotic-selectable marker in chromosomal mutagenesis of the essential gene *asd* and *dapB* of *Burkholderia pseudomallei*. *Appl Environ Microbiol* **75**: 6062–6075.

Padgett, S.R., Kolacz, K., Delannay, X., Re, D., LaVallee, B., Tinius, C., et al. (1995) Development, identification, and characterization of a glyphosate-resistant soybean line. *Crop Sci* **35**: 1451–1461.

Padgett, S.R., Re, D.B., Gasser, C.S., Eichholtz, D.A., Frazier, R.B., Hironaka, C.M., et al. (1991) Site-directed mutagenesis of a conserved region of the 5-enolpyruvylshikimate-3-phosphate synthase active site. *J Biol Chem* **266**: 22364–22369.

Pan, L., Yu, Q., Han, H., Mao, L., Nyporko, A., Fan, L.J., et al. (2019) Alido-keto reductase metabolizes glyphosate and confers glyphosate resistance in *Echinocloa colona*. *Plant Physiol* **181**: 1519–1534.

Park, H., Hilsenbeck, J.L., Kim, H.J., Shuttleworth, W.A., Park, Y.H., Evans, J.N., and Kang, C.H. (2004) Structural studies of *Streptococcus pneumoniae* EPSP synthase in unliganded state. Tetrahedral intermediate-bound state and S3P-GLP-bound state. *Mol Microbiol* **51**: 963–971.

Pedotti, M., Ghisla, S., Motteran, L., Molla, G., and Pollegioni, L. (2009b) Catalytic and redox properties of glycine oxidase from *Bacillus subtilis*. *Biochimie* **91**: 604–612.
Evolution of microbial glyphosate resistance

Rao, R.N., Allen, N.E., Hobbs, J.N., Savino, C., Vallen, B., and Pollegioni, L. (2009a) Glyphosate resistance by engineering the flavoenzyme glycine oxidase. J Biol Chem 284: 36415–36423.

Penaloza-Vazquez, A., Mena, G.L., Herrera-Estrella, L., and Bailey, A.M. (1995) Cloning and sequencing of the genes involved in glyphosate utilization by Pseudomonas pseudomallei. Appl Environ Microbiol 61: 538–543.

Peng, Y., Abercrombie, L.L.G., Yuan, J.S., Riggins, C.W., Sammons, R.D., Trelan, P.J., and Stewart, C.N. (2010) Characterization of the horseweed (Conyza canadensis) transcriptome using GS-FLX 454 pyrosequencing and its application for expression analysis of candidate non-target herbicide resistance genes. Pest Manag Sci 66: 1053–1062.

Pipke, R., and Amrhein, N. (1988a) Degradation of the phosphonate herbicide glyphosate by Arthrobacter atrovunicans ATCC 13752. Appl Environ Microbiol 54: 1293–1296.

Pipke, R., and Amrhein, N. (1988b) Isolation and characterization of a mutant of Arthrobacter sp. strain GLP-1 which utilizes the herbicide glyphosate as its sole source of phosphorus and nitrogen. Appl Environ Microbiol 54: 2868–2870.

Pipke, R., Amrhein, N., Jacob, G.S., Schulz, A., and Amrhein, N. (1987a) Uptake of glyphosate by an Arthrobacter sp. Appl Environ Microbiol 53: 974–978.

Pollegioni, L., Schönbrunn, E., and Siehl, D. (2011) Molecular basis of glyphosate resistance – different approaches through protein engineering. FEBS J 278: 2753–2766.

Pöpke, J., Bote, K., Ramesh, A., Murugaiyan, J., Kuropka, B., Köhl, M., et al. (2020) Selection for resistance to a glyphosate-containing herbicide in Salmonella enterica does not result in a sustained activation of the resistance response of increased cross-resistance and cross-resistance to clinically important antibiotics. Appl Environ Microbiol 86: e01204–e01220.

Prietman, M.A., Healy, M.L., Becker, A., Alberg, D.G., Barlett, P.A., Lushington, G.H., and Schönbrenn, E. (2005) Interaction of phosphonate analogues of the tetrahydrofolate reductase intermediate with 5-enolpyruvylshikimate-3-phosphate synthase in atomic detail. Biochemistry 44: 3241–3248.

Rao, R.N., Allen, N.E., Hobbs, J.N., Albom, W.E., Kirst, H. A., and Paschal, J.W. (1983) Genetic and enzymatic basis of hygromycin B resistance in Escherichia coli. Antimicrob Agents Chemother 24: 689–695.

Regenberg, B., Düring-Olsen, L., Kielland-Brandt, M.C., and Holmberg, S. (1999) Substrate specificity and gene expression of the amino acid permease in Saccharomyces cerevisiae. Curr Genet 36: 317–326.

Regenberg, B., Holmberg, S., Olsen, L.D., and Kielland-Brandt, M.C. (1998) Dip5p mediates high-affinity and high-capacity transport of L-glutamate and L-aspartate in Saccharomyces cerevisiae. Curr Genet 33: 171–177.

Relyea, R.E. (2005) The lethal impact of roundup on aquatic and terrestrial amphibians. Ecol Appl 15: 1118–1124.

Roberts, C.W., Roberts, F., Lyons, R.E., Kirisis, M.J., Mui, E. J., Finnerty, J., et al. (2002) The shikimate pathway and its branches in apicomplexan parasites. J Infect Dis 185: S25–S36.

Roberts, F., Roberts, C.W., Johnson, J.J., Kyle, D.E., Krell, T., Coggins, J.R., et al. (1998) Evidence for the shikimate pathway in apicomplexan parasites. Nature 393: 801–805.

Rogers, B., Decottignies, A., Kolaczkowski, M., Carvajal, E., Balzi, E., and Goffeau, A. (2001) The pleiotropic drug ABC transporters from Saccharomyces cerevisiae. J Mol Microbiol Biotechnol 3: 207–214.

Rogers, S.G., Brand, L.A., Holder, S.B., Sharps, E.S., and Brackin, M.J. (1983) Amplification of the aroA gene from Escherichia coli results in resistance to the herbicide glyphosate. Appl Environ Microbiol 46: 37–43.

Rojano-Delgado, A.M., Cruz-Hipolito, H., De Prado, R., Luque de Castro, M.D., and Franco, A.R. (2012) Limited uptake, translocation and enhanced metabolic degradation contribute to glyphosate resistance in Mucuna pruriens var. utilis plants. Phytochemistry 73: 34–41.

Rong-Mullins, X., Ravishankar, A., McNeal, K.A., Lonergan, Z.R., Biega, A.C., Creamer, J.P., and Gallagher, J.E.G. (2017) Genetic variation in Dip5, an amino acid permease, and Pdr5, a multiple drug transporter, regulates glyphosate resistance in S. cerevisiae. PLoS One 12: e0187522.

Saier, M.H. (2000a) Families of transmembrane transporters selective for amino acids and their derivatives. Microbiology 146: 1775–1795.

Saier, M.H. (2000b) A functional-phylogenetic classification system for transmembrane solute transporters. Mol Microbiol Biol Rev 64: 354–411.

Sammons, R.D., and Gaines, T.A. (2014) Glyphosate resistance: state of knowledge. Pest Manag Sci 70: 1367–1377.

Schlatter, D.C., Yin, C., Hulbert, S., Burke, I., and Paulitz, T. (2017) Impacts of repeated glyphosate use on wheat-associated bacteria are small and depend on use history. Appl Environ Microbiol 83: e01354–e01317.

Schönbrenn, E., Eschenburg, S., Shuttleworth, W.A., Schloss, J.V., Amrhein, N., Evans, J.N., and Kabsch, W. (2001) Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate-3-phosphate synthase in atomic detail. Proc Natl Acad Sci U S A 98: 1376–1380.

Schulz, A., Sost, D., and Amrhein, N. (1984) Insensitivity of 5-enolpyruvylshikimic acid-3-phosphate synthase to glyphosate confers resistance to this herbicide in a strain of Aerobacter aerogenes. Arch Microbiol 137: 121–123.

Schütte, G., Eckerstorfer, M., Rastelli, V., Reichenbecher, W., Restrepo-Vassali, S., Ruohonento-Lehto, M., et al. (2017) Herbicide resistance and biodiversity: agronomic and environmental aspects of genetically modified herbicide-resistant plants. Environ Sci Eur 29: 5.

Settembre, E.C., Dorrestain, P.C., Park, J.H., Augustine, A., and Finnerty, J., Finnerty, J., et al. (2002) The shikimate pathway and its branches in apicomplexan parasites. J Infect Dis 185: S25–S36.

© 2021 The Authors. Environmental Microbiology published by Society for Applied Microbiology and John Wiley & Sons Ltd., Environmental Microbiology, 23, 2891–2905.
Seweryn, P., Van, L.B., Kjeldgaard, M., Russo, C.J., Passmore, L.A., Hove-Jensen, B., et al. (2015) Structural insights into the bacterial carbon-phosphorus lyase machinery. Nature 525: 68–72.

Shames, S.L., Wackett, L.P., LaBarge, M.S., Kuczkowski, R. L., and Walsh, C.T. (1987) Fragmentative and stereochemical isomerization probes for homolytic carbon to phosphorus bond scission catalysed by bacterial carbon-phosphorus lyase. Bioorg Chem 15: 366–373.

Shehata, A.A., Schrödl, W., Aldin, A.A., Hefez, H.M., and Krüger, M. (2013) The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Curr Microbiol 65: 350–358.

Shinabarger, D.L., and Braymer, H.D. (1986) Glyphosate catabolism by Pseudomonas sp. strain PG2982. J Bacteriol 168: 702–707.

Shushkova, T.V., Vinokurova, N.G., Baskunov, B.P., Zelenkova, N.F., Sviridov, A.V., Ermakova, I.T., and Leontievsky, A.A. (2016) Glyphosate acetylation as a specific trait of Achromobacter sp. kg 16 physiology. Appl Microbiol Biotechnol 100: 847–855.

Siehl, D.L., Hertel, R. Hertel

Siehl, D.L., Castle, L.A., Gorton, R., and Keenan, R.J. (2007) The molecular basis of glyphosate resistance by an optimized microbial acetyltransferase. J Biol Chem 282: 11446–11455.

Singh, B.K., and Walker, A. (2006) Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev 30: 428–471.

Slotboom, D.J., Konings, W.N., and Loikema, J.S. (1999) Structural features of the glutamate transporter family. Mol Microbiol Biol Rev 63: 293–307.

Sost, D., and Amrhein, N. (1990) Substitution of Gly-96 to Ala in the 5-enolpyruvylshikimate-3-phosphate synthase of Klebsiella pneumoniae results in a greatly reduced affinity for the herbicide glyphosate. Arch Biochem Biophys 282: 433–436.

Stalker, D.M., Hiatt, W.R., and Comai, L. (1985) A single amino acid substitution in the enzyme 5-enolpyruvylshikimate-3-phosphate synthase confers resistance to the herbicide glyphosate. J Biol Chem 260: 4724–4728.

Staub, J.M., Brand, L., Tran, M., Kong, Y., and Rogers, S.G. (2012) Bacterial glyphosate resistance conferred by overexpression of an E. coli membrane efflux transporter. J Ind Microbiol Biotechnol 39: 641–647.

Steinrücken, H.C., and Amrhein, N. (1980) The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimate acid-3-phosphate synthase. Biochem Biophys Res Commun 94: 1207–1212.

Steinrücken, H.C., and Amrhein, N. (1984) 5-Enolpyruvylshikimate-3-phosphate synthase of Klebsiella pneumoniae. Eur J Biochem 143: 341–349.

Stokstad, E. (2004) Biotechnology. A new tack on herbicide resistance. Science 304: 1089.

Studnik, H., Liebsch, S., Forlani, G., Wieczorek, D., Kafarski, P., and Lipok, J. (2015) Amino polypophosphonates – chemical features and practical use, environmental durability and biodegradation. N Biotechnol 32: 1–6.

Sviridov, A.V., Shushkova, T.V., Ermakova, I.T., Ivanova, E. V., Epiketov, D.O., and Leontievskii, A.A. (2015) Microbial degradation of glyphosate herbicides (review). Prikl Biokhim Mikrobiol 51: 183–190.

Sviridov, A.V., Shushkova, T.V., Zelenkova, N.F., Vinokurova, N.G., Morgunov, I.G., Ermakova, I.T., and Leontievsky, A.A. (2012) Distribution of glyphosate and methylphosphonate catabolism in soil bacteria Ochrobactrum anthropi and Achromobacter sp. Appl Microbiol Biotechnol 93: 787–796.

Svimirov, A.V., Zelenkova, N.F., Vinokurova, N.G., Ermakova, I.T., and Leontievsky, A.A. (2011) New approaches to identification and activity estimation of glyphosate degradation enzymes. Biochemistry (Mosc) 76: 720–725.

Tao, B., Shao, B.H., Qiao, Y.X., Wang, X.Q., Chang, S.J., and Qiu, L.J. (2017) Identification and functional analysis of a new glyphosate resistance gene from a fungus cDNA library. Pestic Biochem Physiol 140: 65–68.

Tincher, C., Long, H., Behringer, M., Walker, N., and Lynch, M. (2017) The glyphosate-based herbicide roundup does not elevate genome-wide mutagenesis of Escherichia coli. G3 (Bethesda) 7: 3331–3335.

Tolner, B., Ubbink-Kok, T., Poolman, B., and Konings, W.N. (1995) Characterization of the proton/glutamate symport protein of Bacillus subtilis and its functional expression in Escherichia coli. J Bacteriol 177: 2863–2869.

VanDrisse, C.M., Hentchel, K.L., and Escalante-Semerena, C.J. (2016) Phosphinothricin acetyltransferases identified using in vivo, in vitro, and bioinformatic analyses. Appl Environ Microbiol 82: 7041–7051.

Vernama, R.S., Vennapusa, A.R., Easwaran, M., Chandrashekar, B.K., Rao, H., Ghanti, K., et al. (2017) Aldo-keto reductase enzymes detoxify glyphosate and improve herbicide resistance in plants. Plant Biotechnol 15: 794–804.

Villarreal-Chiu, J.F., Quinn, J.P., and McGrath, J.W. (2012) The genes and enzymes of phosphonate metabolism in bacteria, and their distribution in the marine environment. Front Microbiol 3: 19.

Whermann, A., Van Vliet, A., Opsomer, C., Bottermann, J., and Schultz, A. (1996) The similarities of bar and pat gene products make them equally applicable for plant engineers. Nat Biotechnol 14: 1274–1278.

Wicke, D., Schulz, L.M., Lentes, S., Scholz, P., Poehlein, A., Gibhardt, J., et al. (2019) Identification of the first glyphosate transporter by genomic adaptation. Environ Microbiol 21: 1287–1305.

Wilson, D.J., Patton, S., Florova, G., Hale, V., and Reynolds, K.A. (1998) The shikimate acid pathway and polyketide biosynthesis. J Ind Microbiol Biotechnol 20: 293–300.

Xu, J., Li, G., Wang, Z., Si, L., He, S., Cai, J., et al. (2016) The role of L-type amino acid transporters in the uptake of glyphosate across mammalian epithelial tissues. Chemosphere 145: 487–494.

Yu, X.M., Yu, T., Yin, G.H., Dong, Q.L., An, M., Wang, H.R., and Ai, C.X. (2015) Glyphosate biodegradation and
potential soil bioremediation by *Bacillus subtilis* strain Bs-15. *Genet Mol Res* 14: 14717–14730.

Yuan, J.S., Abercrombie, L.L.G., Cao, Y., Halfhill, M.D., Peng, Y., Hu, J., et al. (2010) Functional genomics analysis of horseweed (*Coryza canadensis*) with special reference to the evolution of non-target site glyphosate resistance. *Weed Sci* 58: 109–117.

Zaprasis, A., Bleisteiner, M., Kerres, A., Hoffmann, T., and Bremer, E. (2015) Uptake of amino acids and their metabolic conversion into the compatible solute proline confers osmoprotection to *Bacillus subtilis*. *Appl Environ Microbiol* 81: 250–259.

Zaprasis, A., Hoffmann, T., Stannek, L., Gunka, K., Commichau, F.M., and Bremer, E. (2014) The γ-aminobutyrate permease GabP serves as the third proline transporter in *Bacillus subtilis*. *J Bacteriol* 196: 515–526.

Zeleznick, L.D., Myers, T.C., and Titchener, E.B. (1963) Growth of *Escherichia coli* on methyl- and ethylphosphonic acids. *Biochim Biophys Acta* 78: 546–547.

Zhang, Q., and van der Donk, W.A. (2012) Answers to the carbon-phosphorus lyase conundrum. *Chembiochem* 13: 627–629.

Zhang, X.B., Tang, Q.L., Wang, X.J., and Wang, Z.X. (2017) Development of glyphosate-resistant transgenic cotton plants harboring the G2-aroA gene. *J Integr Agric* 16: 551–558.

Zi, X.Y., Yao, J.C., Li, H.W., Huang, Y., and Li, W.J. (2014) Genome-wide identification, domain architectures and phylogenetic analysis provide new insights into the early evolution of shikimate pathway in prokaryotes. *Mol Phylogenet Evol* 75: 154–164.

Zhu, N., Wang, X., Li, D., Lin, Y., You, X., Jiang, J., et al. (2018) IMB-T130 targets 3-dehydroquinate synthase and inhibits *Mycobacterium tuberculosis*. *Sci Rep* 8: 17439.

Zimdahl, R.L. (2010) *A History of Weed Science in the United States*: Elsevier Insights, p. 105. https://www.sciencedirect.com/book/9780123814951/a-history-of-weed-science-in-the-united-states