

S5 Fig. Phylogenetic analysis (A) and conserved amino acids (B) of 5-enolpyruvylshikimate-3-phosphate synthases (EPSPS). (A) amino acid sequences were taken from a previously reported phylogenetic tree [1], and sequences of species phylogenetically related to the isolated strains were selected in GenBank. *E. coli* MurA sequence was used as an outgroup belonging to the EPSPS family. Sequences were aligned using Muscle and trimmed to 726 sites (amino acids 4 to 419 of *V. cholerae* reference sequence). Le and Gascuel model with discrete Gamma distribution and allowance for invariant sites (LG+I+G) was selected as best-fit model of protein evolution using ProtTest [2]. The Bayesian phylogenetic tree was inferred using MrBayes V3.2.6 software [3], with branch probabilities evaluated from 865 000 simulations and 15% burn-in. The consensus phylogenetic tree was built by majority greedy clustering with \geq 0.5 support probability. The glyphosate-sensitive Class I α and Class I α and the glyphosate-resistant Class II EPSPS are indicated in blue, green and red respectively. (B) Presence of the conserved amino acid residues characteristic of Classes I α (blue), I α (green) and II (red) according to Light *et al.* [1] in the representative species mentioned in A. Positions are given for reference sequences from *V. cholerae* (Class II) and *C. burnetii* (Class II). Stars (*) indicate non-conserved amino acids.

References:

- 1. Light SH, Krishna SN, Minasov G, Anderson WF. An unusual cation-binding site and distinct domain-domain interactions distinguish Class II enolpyruvylshikimate-3-phosphate synthases. Biochemistry. 2016;55: 1239-1245.
- 2. Darriba D, Taboada GL, Doallo R, Posada D. ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics. 2011;27: 1164-1165.
- 3. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology. 2012;61: 539-542.