SUPPLEMENTARY MATERIAL

Classification of the glyphosate target enzyme (5-enolpyruvylshikimate-3-phosphate synthase)

Lyydia Leino[1,&], Tuomas Tall[1,&], Marjo Helander[1], Irma Saloniemi[1], Kari Saikkonen[1], Suvi Ruuskanen[1], Pere Puigbò[1,2,*]

[1] *Department of Biology, University of Turku, Turku, Finland*

[2] *Nutrition and Health Unit, Eurecat Technology Centre of Catalonia, Reus, Catalonia, Spain*

[&] *Equal contribution of the authors*

[*] *Corresponding author: pepuav@utu.fi*

SUPPLEMENTARY TABLES

Supplementary table 1. Alignment of EPSPS reference sequences. Conserved amino acids are highlighted in blue (Class Iα), red (Class Iβ), green (Class II), yellow (Class III) and grey (Class IV).

Positions	Class Iα Ref. vcEPSPS	Class Iβ Ref. vcEPSPS	Class II Ref. cbEPSPS	Class III Ref. bvEPSPS	Class IV Ref. sdEPSPS
1	-,-	-,-	-,-	1,M	-,-
2	-,-	-,-	-,-	2,M	-,-
3	-,-	-,-	-,-	3,M	-,-
4	-,-	-,-	-,-	4,G	-,-
5	-,-	-,-	-,-	5,R	-,-
6	1,M	1,M	-,-	6,A	-,-
7	2,E	2,E	1,M	7,K	-,-
8	3,S	3,S	2,D	8,L	-,-
9	4,L	4,L	3,Y	9,T	-,-
10	5,T	5,T	4,Q	10,I	-,-
11	6,L	6,L	5,T	11,I	-,-
12	7,Q	7,Q	6,I	12,P	1,M
13	8,P	8,P	7,P	13,P	2,P
14	9,I	9,I	8,S	14,G	3,V
15	10,E	10,E	9,Q	15,K	4,A
16	11,L	11,L	10,G	16,P	5,D
17	12,I	12,I	11,L	17,L	6,I
18	13,S	13,S	12,S	18,T	-,-
19	14,G	14,G	13,G	19,G	-,-
20	15,E	15,E	14,E	20,R	-,-
21	16,V	16,V	15,I	21,A	-,-
22	17,N	17,N	16,C	22,M	-,-
23	18,L	18,L	17,V	23,P	-,-

24	19,P	19,P	18,P	24,P	7,P
25	20,G	20,G	19,G	25,G	8,G
26	21,S	21,S	20,D	26,S	9,S
27	22,K	22,K	21,K	27,K	10,K
28	23,S	23,S	22,S	28,S	11,S
29	24,V	24,V	23,I	29,I	12,I
30	25,S	25,S	24,S	30,T	13,T
31	26,N	26,N	25,H	31,N	14,A
32	27,R	27,R	26,R	32,R	15,R
33	28,A	28,A	27,A	33,A	16,A
34	29,L	29,L	28,V	34,L	17,L
35	30,L	30,L	29,L	35,L	18,F
36	31,L	31,L	30,L	36,L	19,L
37	32,A	32,A	31,A	37,A	20,A
38	33,A	33,A	32,A	38,G	21,A
39	34,L	34,L	33,I	39,L	22,A
40	35,A	35,A	34,A	40,A	23,A
41	36,S	36,S	35,E	41,K	24,D
42	37,G	37,G	36,G	42,G	25,G
43	38,T	38,T	37,Q	43,T	26,V
44	39,T	39,T	38,T	44,S	27,T
45	40,R	40,R	39,Q	45,R	28,T
46	41,L	41,L	40,V	46,L	29,L
47	42,T	42,T	41,D	47,T	30,V
48	43,N	43,N	42,G	48,G	31,R
49	44,L	44,L	43,F	49,A	32,P
50	45,L	45,L	44,L	50,L	33,L

51	46,D	46,D	45,M	51,K	34,R
52	47,S	47,S	46,G	52,S	35,S
53	48,D	48,D	47,A	53,D	36,D
54	49,D	49,D	48,D	54,D	37,D
55	50,I	50,I	49,N	55,T	38,T
56	51,R	51,R	50,L	56,R	39,E
57	52,H	52,H	51,A	57,Y	40,G
58	53,M	53,M	52,M	58,M	41,F
59	54,L	54,L	53,V	59,A	42,A
60	55,N	55,N	54,S	60,E	43,E
61	56,A	56,A	55,A	61,A	44,G
62	57,L	57,L	56,L	62,L	45,L
63	58,T	58,T	57,Q	63,R	46,A
64	59,K	59,K	58,Q	64,A	47,R
65	60,L	60,L	59,M	65,M	48,L
66	61,G	61,G	60,G	66,G	49,G
67	62,V	62,V	61,A	67,V	50,Y
68	63,N	63,N	62,S	68,T	51,R
69	64,Y	64,Y	63,I	69,I	52,V
70	65,R	65,R	64,Q	70,D	53,G
71	66,L	66,L	65,V	-,-	54,R
72	67,S	67,S	66,I	71,E	55,T
73	68,A	68,A	67,E	72,P	56,P
74	69,D	69,D	68,D	73,D	57,D
75	70,K	70,K	69,E	74,D	-,-
76	71,T	71,T	70,N	75,T	-,-
77	72,T	72,T	71,I	76,T	58,S

78	73,C	73,C	72,L	77,F	59,W
79	74,E	74,E	73,V	78,I	60,Q
80	75,V	75,V	74,V	79,V	61,V
81	76,E	76,E	75,E	80,K	62,D
82	77,G	77,G	76,G	81,G	63,G
83	78,L	78,L	77,V	82,S	64,R
84	79,G	79,G	78,G	83,G	65,P
85	80,Q	80,Q	79,M	84,K	66,Q
86	81,A	81,A	80,T	85,L	-,-
87	82,F	82,F	81,G	86,Q	67,G
88	83,H	83,H	82,L	87,P	68,P
89	84,T	84,T	83,Q	88,P	69,A
90	85,T	85,T	84,A	89,A	70,V
91	86,Q	86,Q	85,P	90,A	71,A
92	87,P	87,P	86,P	91,P	72,E
93	88,L	88,L	87,E	-,-	73,A
94	89,E	89,E	88,A	-,-	74,D
95	90,L	90,L	89,L	92,L	75,V
96	91,F	91,F	90,D	93,F	76,Y
97	92,L	92,L	91,C	94,L	77,C
98	93,G	93,G	92,G	95,G	78,R
99	94,N	94,N	93,N	96,N	79,D
100	95,A	95,A	94,S	97,A	80,G
101	96,G	96,G	95,G	98,G	81,A
102	97,T	97,T	96,T	99,T	82,T
103	98,A	98,A	97,A	100,A	83,T
104	99,M	99,M	98,I	101,T	84,A

105	100,R	100,R	99,R	102,R	85,R
106	101,P	101,P	100,L	103,F	86,F
107	102,L	102,L	101,L	104,L	87,L
108	103,A	103,A	102,S	105,T	88,P
109	104,A	104,A	103,G	106,A	89,T
110	105,A	105,A	104,L	107,A	90,L
111	106,L	106,L	105,L	108,A	91,A
112	107,C	107,C	106,A	109,A	92,A
113	108,L	108,L	107,G	110,L	93,A
114	109,G	109,G	108,Q	111,V	94,G
115	110,Q	110,Q	109,P	112,D	95,H
116	111,G	111,G	110,F	113,G	96,G
117	112,D	112,D	111,N	114,K	97,T
118	113,Y	113,Y	112,T	115,V	98,Y
119	114,V	114,V	113,V	116,I	99,R
120	115,L	115,L	114,L	117,V	100,F
121	116,T	116,T	115,T	118,D	101,D
122	117,G	117,G	116,G	119,G	102,A
123	118,E	118,E	117,D	120,D	103,S
124	119,P	119,P	118,S	121,A	104,E
125	120,R	120,R	119,S	122,H	105,Q
126	121,M	121,M	120,L	123,M	106,M
127	122,K	122,K	121,Q	124,R	107,R
128	123,E	123,E	122,R	125,K	108,R
129	124,R	124,R	123,R	126,R	109,R
130	125,P	125,P	124,P	127,P	110,P
131	126,I	126,I	125,M	128,I	111,L

132	127,G	127,G	126,K	129,G	112,L
133	128,H	128,H	127,R	130,P	113,P
134	129,L	129,L	128,I	131,L	114,L
135	130,V	130,V	129,I	132,V	115,T
136	131,D	131,D	130,D	133,D	116,R
137	132,A	132,A	131,P	134,A	117,A
138	133,L	133,L	132,L	135,L	118,L
139	134,R	134,R	133,T	136,R	119,R
140	135,Q	135,Q	134,L	137,S	120,E
141	136,A	136,A	135,M	138,L	121,L
142	137,G	137,G	136,G	139,G	122,G
143	138,A	138,A	137,A	140,I	123,V
144	139,Q	139,Q	138,K	141,D	124,D
145	140,I	140,I	139,I	142,A	125,L
146	141,E	141,E	140,D	143,S	126,R
147	142,Y	142,Y	141,S	144,A	127,H
148	143,L	143,L	142,T	145,E	128,E
149	144,E	144,E	143,G	146,T	129,E
150	145,Q	145,Q	-,-	-,-	130,R
151	146,E	146,E	-,-	-,-	131,D
152	147,N	147,N	144,N	147,G	132,G
153	148,F	148,F	145,V	148,C	133,H
154	149,P	149,P	146,P	149,P	134,H
155	150,P	150,P	147,P	150,P	135,P
156	151,L	151,L	148,L	151,V	136,L
157	152,R	152,R	149,K	152,T	137,T
158	153,I	153,I	150,I	153,I	138,V

159	154,Q	154,Q	151,Y	154,N	139,R
160	155,G	155,G	152,G	155,G	140,A
161	156,T	156,T	153,N	156,T	141,A
162	157,G	157,G	154,P	157,G	142,G
163	-,-	-,-	155,R	158,R	-,-
164	158,L	158,L	156,L	159,F	143,V
165	159,Q	159,Q	157,T	160,E	144,A
166	160,A	160,A	158,G	161,A	145,G
167	161,G	161,G	159,I	162,S	146,G
168	162,T	162,T	160,H	163,R	147,E
169	163,V	163,V	161,Y	164,V	148,V
170	164,T	164,T	162,Q	165,Q	149,T
171	165,I	165,I	163,L	166,I	150,L
172	166,D	166,D	164,P	167,D	151,D
173	167,G	167,G	165,M	168,G	152,A
174	168,S	168,S	166,A	169,G	153,G
175	169,I	169,I	-,-	170,L	154,Q
176	170,S	170,S	167,S	171,S	155,S
177	171,S	171,S	168,A	172,S	156,S
178	172,Q	172,Q	169,Q	173,Q	157,Q
179	173,F	173,F	170,V	174,Y	158,Y
180	174,L	174,L	171,K	175,V	159,L
181	175,T	175,T	172,S	176,S	160,T
182	176,A	176,A	173,C	177,A	161,A
183	177,F	177,F	174,L	178,L	162,L
184	178,L	178,L	175,L	179,L	163,L
185	179,M	179,M	176,L	180,M	164,L
186	180,S	180,S	177,A	181,M	165,L

187	181,A	181,A	178,G	182,A	166,G
188	182,P	182,P	179,L	183,A	167,P
189	183,L	183,L	180,Y	184,G	168,L
190	184,A	184,A	181,A	185,G	169,T
191	185,Q	185,Q	182,R	186,D	170,E
192	186,G	186,G	183,G	187,R	171,K
193	187,K	187,K	184,K	188,A	172,G
194	188,V	188,V	185,T	189,V	173,L
195	189,T	189,T	186,C	190,D	174,R
196	190,I	190,I	187,I	191,V	175,I
197	191,K	191,K	188,T	192,E	176,H
198	192,I	192,I	189,E	193,L	177,V
199	193,V	193,V	-,-	194,L	178,T
200	194,G	194,G	-,-	195,G	-,-
201	195,E	195,E	-,-	196,E	179,D
202	196,L	196,L	-,-	197,H	180,L
203	197,V	197,V	-,-	198,I	181,V
204	-,-	-,-	-,-	199,G	-,-
205	198,S	198,S	190,P	200,A	182,S
206	199,K	199,K	191,A	201,L	183,V
207	200,P	200,P	192,P	202,G	184,P
208	201,Y	201,Y	193,S	203,Y	185,Y
209	202,I	202,I	194,R	204,I	186,I
210	203,D	203,D	195,D	205,D	187,E
211	204,I	204,I	196,H	206,L	188,I
212	205,T	205,T	197,T	207,T	189,T
213	206,L	206,L	198,E	208,V	190,L

214	207,H	207,H	199,R	209,A	191,A
215	208,I	208,I	200,L	210,A	192,M
216	209,M	209,M	201,L	211,M	193,M
217	210,E	210,E	202,K	212,R	194,R
218	211,Q	211,Q	203,H	213,A	195,A
219	212,F	212,F	204,F	214,F	196,F
220	213,G	213,G	205,H	215,G	197,G
221	214,V	214,V	206,Y	216,A	198,V
222	215,Q	215,Q	207,T	217,K	199,E
223	216,V	216,V	208,L	218,V	200,V
224	217,I	217,I	209,Q	219,E	201,T
225	218,N	218,N	210,K	220,R	202,R
226	219,H	219,H	211,D	221,V	203,E
227	220,D	220,D	212,K	222,S	204,G
228	221,Y	221,Y	213,Q	223,P	205,H
229	222,Q	222,Q	-,-	224,V	-,-
230	223,E	223,E	214,S	225,A	206,D
231	224,F	224,F	215,I	226,W	207,F
232	225,V	225,V	216,C	227,R	208,V
233	226,I	226,I	217,V	228,V	209,V
234	227,P	227,P	218,S	229,E	210,P
235	228,A	228,A	219,G	230,P	211,P
236	229,G	229,G	220,G	231,T	212,G
237	230,Q	230,Q	221,G	232,G	213,G
238	231,S	231,S	222,K	-,-	-,-
239	232,Y	232,Y	223,L	233,Y	214,Y
240	233,V	233,V	-,-	-,-	-,-

241	234,S	234,S	224,K	234,H	215,R
242	235,P	235,P	225,A	235,A	216,A
243	236,G	236,G	226,N	236,A	217,T
244	237,Q	237,Q	227,D	237,D	218,T
245	238,F	238,F	228,I	238,F	219,Y
246	239,L	239,L	229,S	239,V	220,A
247	240,V	240,V	230,I	240,I	221,I
248	241,E	241,E	231,P	241,E	222,E
249	242,G	242,G	232,G	242,P	223,P
250	243,D	243,D	233,D	243,D	224,D
251	244,A	244,A	234,I	244,A	225,A
252	245,S	245,S	235,S	245,S	226,S
253	246,S	246,S	236,S	246,A	227,T
254	247,A	247,A	237,A	247,A	228,S
255	248,S	248,S	238,A	248,T	229,S
256	249,Y	249,Y	239,F	249,Y	230,Y
257	250,F	250,F	240,F	250,L	231,F
258	251,L	251,L	241,I	251,W	232,F
259	252,A	252,A	242,V	252,A	233,A
260	253,A	253,A	243,A	253,A	234,A
261	254,A	254,A	244,A	254,E	235,A
262	255,A	255,A	245,T	255,V	236,A
263	256,I	256,I	246,I	256,L	237,L
264	257,K	257,K	247,T	257,S	238,S
265	-,-	-,-	248,P	-,-	-,-
266	258,G	258,G	249,G	258,G	239,G
267	259,G	259,G	250,S	259,G	240,G

268	260,E	260,E	251,A	260,K	241,E
269	261,V	261,V	252,I	261,I	242,V
270	262,K	262,K	253,R	262,D	243,T
271	263,V	263,V	254,L	263,L	244,V
272	264,T	264,T	255,C	264,G	245,P
273	265,G	265,G	256,R	265,T	246,G
274	266,I	266,I	257,V	266,P	247,L
275	267,G	267,G	258,G	267,A	248,G
276	268,K	268,K	259,V	268,E	249,E
277	269,N	269,N	260,N	269,Q	250,G
278	270,S	270,S	261,P	270,F	251,A
279	271,I	271,I	262,T	271,S	252,L
280	272,Q	272,Q	263,R	272,Q	253,Q
281	273,G	273,G	-,-	273,P	254,G
282	274,D	274,D	-,-	274,D	255,D
283	275,I	275,I	264,L	275,A	256,L
284	276,Q	276,Q	265,G	276,K	257,G
285	277,F	277,F	266,V	277,A	258,F
286	278,A	278,A	267,I	278,Y	259,V
287	279,D	279,D	268,N	279,D	260,D
288	280,A	280,A	269,L	280,L	261,V
289	281,L	281,L	270,L	281,I	262,L
290	282,E	282,E	271,K	282,S	263,R
291	283,K	283,K	272,M	283,K	264,R
292	284,M	284,M	273,M	-,-	265,M
293	285,G	285,G	274,G	-,-	266,G
294	286,A	286,A	275,A	-,-	267,A

295	287,Q	287,Q	276,D	-,-	268,E
296	288,I	288,I	277,I	-,-	269,V
297	289,E	289,E	278,E	-,-	270,E
298	290,W	290,W	279,V	-,-	271,I
299	291,G	291,G	280,T	-,-	272,G
300	-,-	-,-	281,H	-,-	-,-
301	-,-	-,-	282,Y	-,-	-,-
302	-,-	-,-	283,T	-,-	-,-
303	-,-	-,-	284,E	-,-	-,-
304	-,-	-,-	285,K	-,-	-,-
305	-,-	-,-	286,N	-,-	-,-
306	-,-	-,-	287,E	-,-	-,-
307	-,-	-,-	288,E	-,-	-,-
308	-,-	-,-	289,P	-,-	-,-
309	292,D	292,D	290,T	-,-	273,A
310	293,D	293,D	291,A	-,-	274,D
311	294,Y	294,Y	292,D	-,-	275,R
312	295,V	295,V	293,I	-,-	276,T
313	296,I	296,I	294,T	-,-	277,T
314	297,A	297,A	295,V	-,-	278,V
315	298,R	298,R	296,R	-,-	279,R
316	-,-	-,-	-,-	-,-	280,G
317	299,R	299,R	297,H	284,F	281,T
318	300,G	300,G	298,A	285,P	282,G
319	301,E	301,E	299,R	286,H	283,E
320	302,L	302,L	300,L	287,L	284,L
321	303,N	303,N	301,K	288,P	285,R
322	304,A	304,A	302,G	289,A	286,G

323	305,V	305,V	303,I	290,V	287,L
324	306,D	306,D	304,D	-,-	288,T
325	307,L	307,L	305,I	291,I	289,V
326	308,D	308,D	306,P	292,D	290,N
327	309,F	309,F	307,P	293,G	291,M
328	310,N	310,N	308,D	294,S	292,R
329	311,H	311,H	309,Q	295,Q	293,D
330	312,I	312,I	310,V	296,M	294,I
331	313,P	313,P	311,P	297,Q	295,S
332	-,-	-,-	312,L	-,-	-,-
333	-,-	-,-	313,T	-,-	-,-
334	-,-	-,-	314,I	-,-	-,-
335	314,D	314,D	315,D	298,D	296,D
336	315,A	315,A	316,E	299,A	297,T
337	316,A	316,A	317,F	300,I	298,M
338	317,M	317,M	318,P	301,P	299,P
339	318,T	318,T	319,V	302,T	300,T
340	319,I	319,I	320,L	303,L	301,L
341	320,A	320,A	321,L	304,A	302,A
342	321,T	321,T	322,I	305,V	303,A
343	322,T	322,T	323,A	306,L	304,I
344	323,A	323,A	324,A	307,A	305,A
345	324,L	324,L	325,A	308,A	306,P
346	325,F	325,F	326,V	309,F	307,F
347	326,A	326,A	327,A	310,N	308,A
348	327,K	327,K	328,Q	311,E	309,S
349	328,G	328,G	329,G	312,M	310,G

350	329,T	329,T	330,K	313,P	311,P
351	330,T	330,T	331,T	314,V	312,V
352	331,A	331,A	332,V	315,R	313,R
353	332,I	332,I	333,L	316,F	314,I
354	333,R	333,R	334,R	317,V	315,E
355	334,N	334,N	335,D	318,G	316,D
356	335,V	335,V	336,A	319,I	317,V
357	336,Y	336,Y	337,A	320,E	318,A
358	337,N	337,N	338,E	321,N	319,N
359	338,W	338,W	339,L	322,L	320,T
360	339,R	339,R	340,R	323,R	321,R
361	340,V	340,V	341,V	324,V	322,V
362	341,K	341,K	342,K	325,K	323,K
363	342,E	342,E	343,E	326,E	324,E
364	343,T	343,T	344,T	327,C	325,C
365	344,D	344,D	345,D	328,D	326,D
366	345,R	345,R	346,R	329,R	327,R
367	346,L	346,L	347,I	330,I	328,L
368	347,A	347,A	348,A	331,R	329,E
369	348,A	348,A	349,A	332,A	330,A
370	349,M	349,M	350,M	333,L	331,C
371	350,A	350,A	351,V	334,S	332,A
372	351,T	351,T	352,D	335,S	333,E
373	352,E	352,E	353,G	336,G	334,N
374	353,L	353,L	354,L	337,L	335,L
375	354,R	354,R	355,Q	338,S	336,R
376	355,K	355,K	356,K	339,R	337,R

377	356,V	356,V	357,L	340,I	338,L
378	-,-	-,-	-,-	341,V	-,-
379	-,-	-,-	-,-	342,P	-,-
380	357,G	357,G	358,G	343,N	339,G
381	358,A	358,A	359,I	344,L	340,V
382	359,T	359,T	360,A	345,G	341,R
383	360,V	360,V	361,A	346,T	342,V
384	361,E	361,E	362,E	347,E	343,E
385	362,E	362,E	363,S	348,E	344,T
386	363,G	363,G	364,L	349,G	345,G
387	364,E	364,E	365,P	350,D	346,P
388	365,D	365,D	366,D	351,D	347,D
389	366,F	366,F	367,G	352,L	348,W
390	367,I	367,I	368,V	353,I	349,I
391	368,V	368,V	369,I	354,I	350,E
392	369,I	369,I	370,I	355,A	351,I
393	370,T	370,T	371,Q	356,S	352,H
394	371,P	371,P	372,G	357,D	353,P
395	372,P	372,P	373,G	358,P	354,G
396	373,T	373,T	374,T	359,S	355,A
397	-,-	-,-	-,-	360,L	-,-
398	-,-	-,-	-,-	361,A	-,-
399	-,-	-,-	-,-	362,G	-,-
400	374,K	374,K	-,-	363,K	356,T
401	375,L	375,L	375,L	364,I	357,P
402	376,I	376,I	376,E	365,L	358,T
403	377,H	377,H	377,G	366,T	359,G

404	378,A	378,A	378,G	367,A	360,A
405	379,A	379,A	379,E	368,E	361,E
406	380,I	380,I	380,V	369,I	362,I
407	381,D	381,D	381,N	370,D	363,K
408	382,T	382,T	382,S	371,S	364,T
409	383,Y	383,Y	383,Y	372,F	365,Y
410	384,D	384,D	384,D	373,A	366,G
411	385,D	385,D	385,D	374,D	367,D
412	386,H	386,H	386,H	375,H	368,H
413	387,R	387,R	387,R	376,R	369,R
414	388,M	388,M	388,I	377,I	370,I
415	389,A	389,A	389,A	378,A	371,V
416	390,M	390,M	390,M	379,M	372,M
417	391,C	391,C	391,A	380,S	373,S
418	392,F	392,F	392,F	381,F	374,F
419	393,S	393,S	393,A	382,A	375,A
420	394,L	394,L	394,V	383,L	376,V
421	395,V	395,V	395,A	384,A	377,T
422	396,A	396,A	396,G	385,G	378,G
423	-,-	-,-	397,T	-,-	-,-
424	397,L	397,L	398,L	386,L	379,L
425	398,S	398,S	399,A	387,K	380,R
426	399,D	399,D	400,K	388,I	381,V
427	400,T	400,T	401,G	389,G	382,P
428	401,P	401,P	402,P	390,G	383,G
429	402,V	402,V	403,V	391,I	384,I
430	403,T	403,T	404,R	392,T	385,S

431	404,I	404,I	405,I	393,I	386,F
432	405,N	405,N	406,R	394,L	387,D
433	406,D	406,D	407,N	395,D	388,D
434	407,P	407,P	408,C	396,P	389,P
435	408,K	408,K	409,D	397,D	390,G
436	409,C	409,C	410,N	398,C	391,C
437	410,T	410,T	411,V	399,V	392,V
438	411,S	411,S	412,K	400,A	393,R
439	412,K	412,K	413,T	401,K	394,K
440	413,T	413,T	414,S	402,T	395,T
441	414,F	414,F	415,F	403,F	396,F
442	415,P	415,P	416,P	404,P	397,P
443	416,D	416,D	417,N	405,S	398,G
444	417,Y	417,Y	418,F	406,Y	399,F
445	418,F	418,F	419,V	407,W	400,H
446	419,D	419,D	420,E	408,N	401,E
447	420,K	420,K	421,L	409,V	402,E
448	421,F	421,F	422,A	410,L	403,F
449	422,A	422,A	423,N	411,S	404,G
450	423,Q	423,Q	424,E	412,S	405,A
451	424,L	424,L	425,V	413,L	406,L
452	425,S	425,S	426,G	414,G	407,R
453	426,R	426,R	427,M	415,V	408,A
454	-,-	-,-	428,N	416,A	409,R
455	-,-	-,-	429,V	417,Y	410,L
456	-,-	-,-	430,K	418,E	-,-
457	-,-	-,-	431,G	419,D	-,-

458	-,-	-,-	432,V	-,-	-,-
459	-,-	-,-	433,R	-,-	-,-
460	-,-	-,-	434,G	-,-	-,-
461	-,-	-,-	435,R	-,-	-,-
462	-,-	-,-	436,G	-,-	-,-
463	-,-	-,-	437,G	-,-	-,-
464	-,-	-,-	438,F	-,-	-,-

Supplementary table 2. Class I-IV EPSPS reference sequences

```
>Vibrio cholerae|vbEPSPS
MESLTLQPIELISGEVNLPGSKSVSNRALLLAALASGTTRLTNLLDSDDIRHMLNALTKL
GVNYRLSADKTTCEVEGLGQAFHTTQPLELFLGNAGTAMRPLAAALCLGQGDYVLTGEPR
MKERPIGHLVDALRQAGAQIEYLEQENFPPLRIQGTGLQAGTVTIDGSISSQFLTAFLMS
APLAQGKVTIKIVGELVSKPYIDITLHIMEQFGVQVINHDYQEFVIPAGQSYVSPGQFLV
EGDASSASYFLAAAAIKGGEVKVTGIGKNSIQGDIQFADALEKMGAQIEWGDDYVIARRG
ELNAVDLDFNHIPDAAMTIATTALFAKGTTAIRNVYNWRVKETDRLAAMATELRKVGATV
EEGEDFIVITPPTKLIHAAIDTYDDHRMAMCFSLVALSDTPVTINDPKCTSKTFPDYFDK
FAQLSR
```

```
>Coxiella burnetii|cbESPSP
MDYQTIPSQGLSGEICVPGDKSISHRAVLLAAIAEGQTQVDGFLMGADNLAMVSALQQMG
ASIQVIEDENILVVEGVGMTGLQAPPEALDCGNSGTAIRLLSGLLAGQPFNTVLTGDSSL
QRRPMKRIIDPLTLMGAKIDSTGNVPPLKIYGNPRLTGIHYQLPMASAQVKSCLLLAGLY
ARGKTCITEPAPSRDHTERLLKHFHYTLQKDKQSICVSGGGKLKANDISIPGDISSAAFF
IVAATITPGSAIRLCRVGVNPTRLGVINLLKMMGADIEVTHYTEKNEEPTADITVRHARL
KGIDIPPDQVPLTIDEFPVLLIAAAVAQGKTVLRDAAELRVKETDRIAAMVDGLQKLGIA
AESLPDGVIIQGGTLEGGEVNSYDDHRIAMAFAVAGTLAKGPVRIRNCDNVKTSFPNFVE
LANEVGMNVKGVRGRGGF
```

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>Brevundimonas vesicularis|bvEPSPS
MMMGRAKLTIIPPGKPLTGRAMPPGSKSITNRALLLAGLAKGTSRLTGALKSDDTRYMAE
ALRAMGVTIDEPDDTTFIVKGSGKLQPPAAPLFLGNAGTATRFLTAAAALVDGKVIVDGD
AHMRKRPIGPLVDALRSLGIDASAETGCPPVTINGTGRFEASRVQIDGGLSSQYVSALLM
MAAGGDRAVDVELLGEHIGALGYIDLTVAAMRAFGAKVERVSPVAWRVEPTGYHAADFVI
EPDASAATYLWAAEVLSGGKIDLGTPAEQFSQPDAKAYDLISKFPHLPAVIDGSQMQDAI
PTLAVLAAFNEMPVRFVGIENLRVKECDRIRALSSGLSRIVPNLGTEEGDDLIIASDPSL
AGKILTAEIDSFADHRIAMSFALAGLKIGGITILDPDCVAKTFPSYWNVLSSLGVAYED
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>Streptomyces davawensis|sdEPSPS
MPVADIPGSKSITARALFLAAAADGVTTLVRPLRSDDTEGFAEGLARLGYRVGRTPDSWQ
VDGRPQGPAVAEADVYCRDGATTARFLPTLAAAGHGTYRFDASEQMRRRPLLPLTRALRE
LGVDLRHEERDGHHPLTVRAAGVAGGEVTLDAGQSSQYLTALLLLGPLTEKGLRIHVTDL
VSVPYIEITLAMMRAFGVEVTREGHDFVVPPGGYRATTYAIEPDASTSSYFFAAAALSGG
EVTVPGLGEGALQGDLGFVDVLRRMGAEVEIGADRTTVRGTGELRGLTVNMRDISDTMPT
LAAIAPFASGPVRIEDVANTRVKECDRLEACAENLRRLGVRVETGPDWIEIHPGATPTGA
EIKTYGDHRIVMSFAVTGLRVPGISFDDPGCVRKTFPGFHEEFGALRARL
```

Supplementary table 3 . Taxonomic distribution of EPSPS proteins by class. EPSPS sequences were obtained from Pfam database [1] .

Taxonomy	Class Iα	Class Iβ	Class II	Class III	Class IV	None
Archaea	15	142	0	4	0	35
Bacteria	1511	1460	1376	217	39	5447
Eukaryota	469	488	0	7	0	123
Acidobacteria	1	1	10	0	0	13
Actinobacteria	490	79	17	16	34	953
Alveolata	6	10	0	0	0	25
Amoebozoa	1	1	0	0	0	0
Aquificae	0	0	12	0	0	14
Armatimonadetes	2	0	1	1	0	5
Asgard group	0	1	0	0	0	0
Bacteroidetes	372	419	4	20	3	421
Caldiserica	0	0	0	0	0	1
Calditrichaeota	0	0	0	0	0	3
Chlamydiae	1	9	0	0	0	19
Chlorobi	0	0	11	0	0	15
Chloroflexi	4	10	6	3	0	45
Choanoflagellida	2	2	0	0	0	0
Chrysiogenetes	0	0	1	0	0	1
Crenarchaeota	0	21	0	2	0	9
Cryptophyta	1	1	0	0	0	0
Cyanobacteria	8	8	98	1	0	118
Deferribacteres	0	0	5	1	0	5
Deinococcus Thermus	2	12	3	1	2	17
Dictyoglomi	0	0	1	0	0	1
Elusimicrobia	0	0	1	0	0	4

Environmental samples	0	4	0	0	0	0
Euryarchaeota	14	99	0	1	0	27
Fibrobacteres	1	3	0	0	0	5
Firmicutes	28	236	440	21	0	1909
Fungi	358	368	0	6	0	57
Fusobacteria	1	14	0	0	0	21
Gemmatimonadetes	1	1	3	0	0	12
Haloplasmatales	0	0	2	0	0	1
Haptophyceae	0	0	0	0	0	4
Ichthyosporea	2	2	0	0	0	0
Ignavibacteriae	0	0	2	0	0	2
Kiritimatiellaeota	0	0	1	0	0	1
Lentisphaerae	1	1	0	0	0	1
Metazoa	0	0	0	0	0	8
Nitrospinae Tectomicrobia group	1	1	0	1	0	6
Nitrospirae	1	2	11	0	0	22
Nucleariidae & Fonticula group	0	1	0	0	0	0
Planctomycetes	20	22	0	8	0	17
Proteobacteria	549	592	714	149	0	1705
Rhizaria	1	1	0	1	0	0
Rhodophyta	2	2	0	0	0	0
Spirochaetes	2	18	4	1	0	61
Stramenopiles	22	23	0	0	0	3
Synergistetes	0	4	7	0	0	14
Tenericutes	0	4	0	0	0	7
Thaumarchaeota	0	7	0	0	0	5
Thermobaculum	0	0	1	1	0	1
Thermodesulfobacteria	1	2	0	1	0	4

Thermotogae	0	2	5	0	0	15
Unclassified Parcubacteria group	2	3	0	0	0	100
Verrucomicrobia	10	1	7	1	0	19
Viridiplantae	74	77	0	0	0	68

Supplementary table 4 . Taxonomic distribution of EPSPS proteins in archaea and bacteria species by Class. EPSPS sequences were obtained from the COG database [2].

Taxonomy	**Class Iα**	**Class Iβ**	**Class II**	**Class III**	**Class IV**	**None**
Archaea	**5**	**63**	**0**	**2**	**0**	**13**
Crenarchaeota	0	10	0	1	0	5
Euryarchaeota	5	50	0	1	0	6
Korarchaeota	0	0	0	0	0	1
Thaumarchaeota	0	3	0	0	0	1
Bacteria	**234**	**223**	**252**	**26**	**2**	**64**
Acidobacteria	0	0	6	0	0	0
Actinobacteria	0	0	0	0	0	0
Aquificae	0	0	8	0	0	0
Bacteroidetes	37	44	2	0	0	7
Chlamydiae	0	5	0	0	0	1
Chlorobi	0	0	0	0	0	0
Chloroflexi	2	4	3	2	0	2
Cyanobacteria	3	3	29	0	0	0
Deinococcus-thermus	2	2	3	1	0	0
Firmicutes (Bacilli)	3	2	25	1	0	4
Firmicutes (Clostridia)	0	13	28	1	0	4
Firmicutes (Mollicutes)	0	0	0	0	0	1
Firmicutes (Other)	0	3	2	0	0	0
Fusobacteria	0	4	0	0	0	0
Other Bacteria	6	3	15	2	0	4
Planctomycetes	6	6	0	3	0	0

Proteobacteria (Alpha)	4	4	57	9	0	7
Proteobacteria (Beta)	47	47	0	2	0	2
Proteobacteria (Delta)	14	17	6	3	0	9
Proteobacteria (Epsilon)	1	1	9	0	0	1
Proteobacteria (Gamma)	52	54	41	0	0	8
Spirochaetes	0	2	1	1	0	3
Synergistetes	0	1	3	0	0	0
Thermotogae	0	0	0	0	0	0

Supplementary table 5. Distributions of EPSPS and EPSPS-associated domains across archaea, bacteria and eukaryotes. Pfam [1] codes of the domains: PF00275 (EPSP_synthase); PF01264 (Chorismate_synt); PF01202 (SKI); PF01487 (DHquinase_I); PF01761 (DHQ_synthase); PF08501 (Shikimate_dh_N); PF01381 (HTH_3); PF01488 (Shikimate_DH); PF02153 (PDH); PF02224 (Cytidylate_kin); PF00501 (PF00501); PF13193 (PF13193); PF13560 (HTH_31); PF01817 (CM_2); PF00623 (RNA_pol_Rpb1_2); PF01885 (PF01885); PF03159 (XRN_N); PF04983 (RNA_pol_Rpb1_3); PF04997 (RNA_pol_Rpb1_1); PF04998 (RNA_pol_Rpb1_5); PF05000 (RNA_pol_Rpb1_4); PF13419 (HAD_2); PF00011 (HSP20); PF00132 (Hexapep); PF00443 (UCH); PF00616 (RasGAP); PF00670 (AdoHcyase_NAD); PF01019 (G_glu_transpept); PF01048 (PNP_UDP_1 and PF01134 (GIDA).

Domains	Archaea	Bacteria	Eukaryota	Fungi	Viridiplantae	Stramenopiles
EPSP_synthase	**181**	**9052**	**623**	**412**	**133**	**26**
Chorismate_synt	1	211	417	374	2	19
SKI	0	126	409	371	1	18
DHquinase_I	0	87	403	368	0	17
DHQ_synthase	0	17	398	368	0	15
Shikimate_dh_N	0	17	155	136	0	14
HTH_3	0	9	2	2	0	0
Shikimate_DH	0	8	2	2	0	0
PDH	0	4	2	2	0	0
Cytidylate_kin	0	4	2	2	0	0

PF00501	0	3	2	2	0	0
PF13193	0	3	2	2	0	0
HTH_31	0	2	2	1	0	0
CM_2	0	1	1	1	0	0
RNA_pol_Rpb1_2	0	1	1	1	0	0
PF01885	0	1	1	1	0	0
XRN_N	0	1	1	1	0	0
RNA_pol_Rpb1_3	0	1	1	1	0	0
RNA_pol_Rpb1_1	0	1	1	1	0	0
RNA_pol_Rpb1_5	0	1	1	1	0	0
RNA_pol_Rpb1_4	0	1	1	1	0	0
HAD_2	0	0	1	1	0	0
HSP20	0	0	1	0	0	0
Hexapep	0	0	1	0	0	0
UCH	0	0	1	0	0	0
RasGAP	0	0	1	0	0	0
AdoHcyase_NAD	0	0	1	0	0	0
G_glu_transpept	0	0	1	0	0	0
PNP_UDP_1	0	0	1	0	0	0
GIDA	0	0	1	0	0	0

Supplementary Table 6. Most frequent EPSPS associated domains in multidomain proteins. FREQ is the frequency of the gene in samples, SP is the number of the gene in how many species they are found and FUNCTION in the description of product and if it is part of shikimate pathway.

GENE	FREQ	SP	FUNCTION
EPSPS	**1448**	**8833**	**6th step of shikimate. pathway, produces EPSP Synthase**
SKI	424	7716	Start of pathway, produces phosphorylates shikimate
DHQ_synthase	420	7429	Second step of pathway, removes a phosphate from DHAP

DHquinase_I	416	2073	3rd step of pathway, produces 3-dehydroquinate dehydratase
Shikimate_dh_N	402	7658	The substrate binding domain of shikimate dehydrogenase
HTH_3	218	9670	Helix-turn-helix, a major structural motif capable of binding DNA
Shikimate_DH	160	6719	4th step. Shikimate / quinate 5-dehydrogenase
PDH	127	7136	Prephenate dehydrogenases are part of Tyrosine biosynthesis.
Cytidylate_kin	88	6874	Kinase of cytidine 5'-monophosphate
PF13193	17	8031	AMP-binding enzyme C-terminal domain for PF00501

Supplementary table 7. List of common bacteria in the gut-microbiome (from supplementary tables 8, 5 and 12 provided by Qin et al. 2010) and their susceptibility to glyphosate. These species or strains are specifically mentioned at Qin et al. (2010). N: Number of sequences; R: Sequences are resistant; S: Sequences are sensitive to glyphosate, U: sequences are unclassified and their sensitiveness is unknown.

Species/strain	**N**	**R / S**	**Class**	**Resistant Sequences (%)**
Actinomyces odontolyticus **ATCC 17982**	1	S	Class I	0%
Anaerofustis stercorihominis **DSM 17244**	1	S	Class I	0%
Anaerotruncus colihominis	5	S	Class I	0%
Bacteroides caccae	5	S	Class I	0%
Bacteroides capillosus	1	S	Class I	0%
Bacteroides cellulosilyticus	4	S	Class I	0%
Bacteroides coprocola	2	S	Class I	0%
Bacteroides coprophilus	1	S	Class I	0%
Bacteroides coprophilus **DSM 18228**	1	S	Class I	0%
Bacteroides dorei	1	S	Class I	0%
Bacteroides dorei **DSM 17855**	1	S	Class I	0%
Bacteroides eggerthii	3	S	Class I	0%
Bacteroides finegoldii	4	S	Class I	0%
Bacteroides fragilis **3_1_12**	1	S	Class I	0%
Bacteroides fragilis **NCTC 9343**	1	S	Class I	0%
Bacteroides fragilis **YCH46**	1	S	Class I	0%
Bacteroides intestinalis	11	S	Class I	0%
Bacteroides ovatus	14	S	Class I	0%
Bacteroides plebeius	11	S	Class I	0%
Bacteroides sp. **2_1_7**	1	S	Class I	0%
Bacteroides sp. **2_2_4**	1	S	Class I	0%

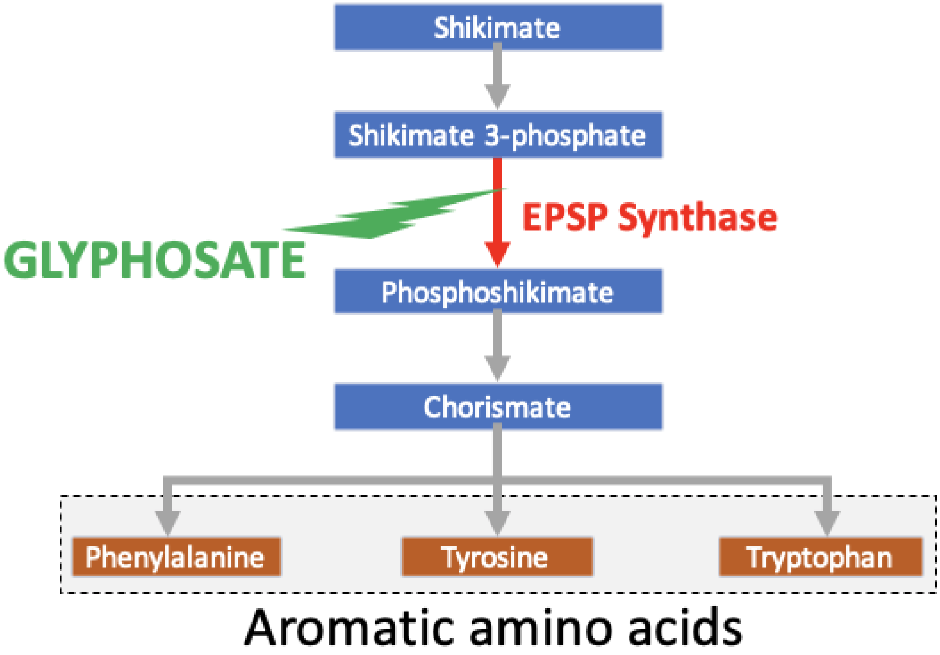
***Bacteroides sp.* 3_2_5**	1	S	Class I	0%
***Bacteroides sp.* 4_3_47FAA**	1	S	Class I	0%
***Bacteroides sp.* 9_1_42FAA**	2	S	Class I	0%
***Bacteroides sp.* D1**	1	S	Class I	0%
Bacteroides stercoris	20	S	Class I	0%
Bacteroides uniformis	10	S	Class I	0%
***Bacteroides vulgatus* ATCC 8482**	1	S	Class I	0%
Bifidobacterium adolescentis	26	25×S 1×R	Class I; Class II	4%
***Bifidobacterium adolescentis* ATCC 15703**	1	S	Class I	0%
***Bifidobacterium adolescentis* L2-32**	1	S	Class I	0%
***Bifidobacterium bifidum* NCIMB 41171**	1	S	Class I	0%
Bifidobacterium breve	27	S	Class I	0%
***Bifidobacterium breve* DSM 20213**	1	S	Class I	0%
Bifidobacterium catenulatum	7	S	Class I	0%
***Bifidobacterium catenulatum* DSM 16992**	1	S	Class I	0%
Bifidobacterium dentium	5	S	Class I	0%
***Bifidobacterium longum* DJO10A**	1	S	Class I	0%
***Bifidobacterium longum* NCC2705**	1	S	Class I	0%
***Bifidobacterium longum subsp. infantis* ATCC 15697**	1	S	Class I	0%
***Bifidobacterium longum subsp. infantis* ATCC 55813**	1	S	Class I	0%
***Bifidobacterium longum subsp. infantis* CCUG 52486**	1	S	Class I	0%
Bifidobacterium pseudocatenulatum	46	45×S 1×U	Class I U	0%
Catenibacterium mitsuokai	3	S	Class I	0%
***Citrobacter sp.* 30_2**	1	S	Class I	0%
***Citrobacter sp.* 30_3**	1	S	Class I	0%
***Citrobacter sp.* 30_4**	1	S	Class I	0%
***Citrobacter sp.* 30_5**	1	S	Class I	0%
***Citrobacter sp.* 30_6**	1	S	Class I	0%
Clostridium leptum	4	S	Class I	0%
***Clostridium leptum* DSM 753**	1	U	U	0%
Clostridium methylpentosum	1	S	Class I	0%
Clostridium nexile	1	S	Class I	0%
Collinsella aerofaciens	9	S	Class I	0%
***Desulfovibrio piger* ATCC 29098**	1	S	Class I	0%
***Enterobacter cancerogenus* ATCC 35316**	1	S	Class I	0%
***Escherichia coli* 536**	1	S	Class I	0%

Escherichia coli APEC O1	1	S	Class I	0%
Escherichia coli ATCC 8739	1	S	Class I	0%
Escherichia coli CFT073	1	S	Class I	0%
Escherichia coli ED1a	1	S	Class I	0%
Escherichia coli HS	1	S	Class I	0%
Escherichia coli IAI39	1	S	Class I	0%
Escherichia coli O127:H6 str. E2348/69	1	S	Class I	0%
Escherichia coli O157:H7 str. EC4115	1	S	Class I	0%
Escherichia coli S88	1	S	Class I	0%
Escherichia coli SE11	1	S	Class I	0%
Escherichia coli SMS-3-5	1	S	Class I	0%
Escherichia coli UTI89	1	S	Class I	0%
Eubacterium siraeum	3	S	Class I	0%
Eubacterium siraeum 70/3	1	S	Class I	0%
Faecalibacterium prausnitzii M21/2	1	S	Class I	0%
Faecalibacterium prausnitzii SL3 3	1	S	Class I	0%
Fusobacterium mortiferum ATCC 9817	1	S	Class I	0%
Fusobacterium sp. 4_1_13	1	S	Class I	0%
Fusobacterium ulcerans ATCC 49185	1	S	Class I	0%
Fusobacterium varium ATCC 27725	1	S	Class I	0%
Holdemania filiformis	1	S	Class I	0%
Methanobrevibacter smithii	6	S	Class I	0%
Methanobrevibacter smithii ATCC 35061	1	S	Class I	0%
Mollicutes bacterium D7	1	S	Class I	0%
Parabacteroides distasonis ATCC 8503	1	S	Class I	0%
Parabacteroides johnsonii	4	S	Class I	0%
Parabacteroides merdae	8	S	Class I	0%
Prevotella copri	27	S	Class I	0%
Providencia alcalifaciens DSM 30120	1	S	Class I	0%
Providencia rustigianii DSM 4541	1	S	Class I	0%
Providencia stuartii ATCC 25827	1	S	Class I	0%
Ruminococcus bromii	13	S	Class I	0%
Ruminococcus bromii L2-63	1	S	Class I	0%
Subdoligranulum variabile	2	S	Class I	0%
Subdoligranulum variabile DSM 15176	1	S	Class I	0%
Alistipes putredinis	3	R	Class III	100%
Anaerobaculum hydrogeniformans ATCC BAA-1850	1	R	Class II	100%
Blautia hansenii	3	R	Class II	100%

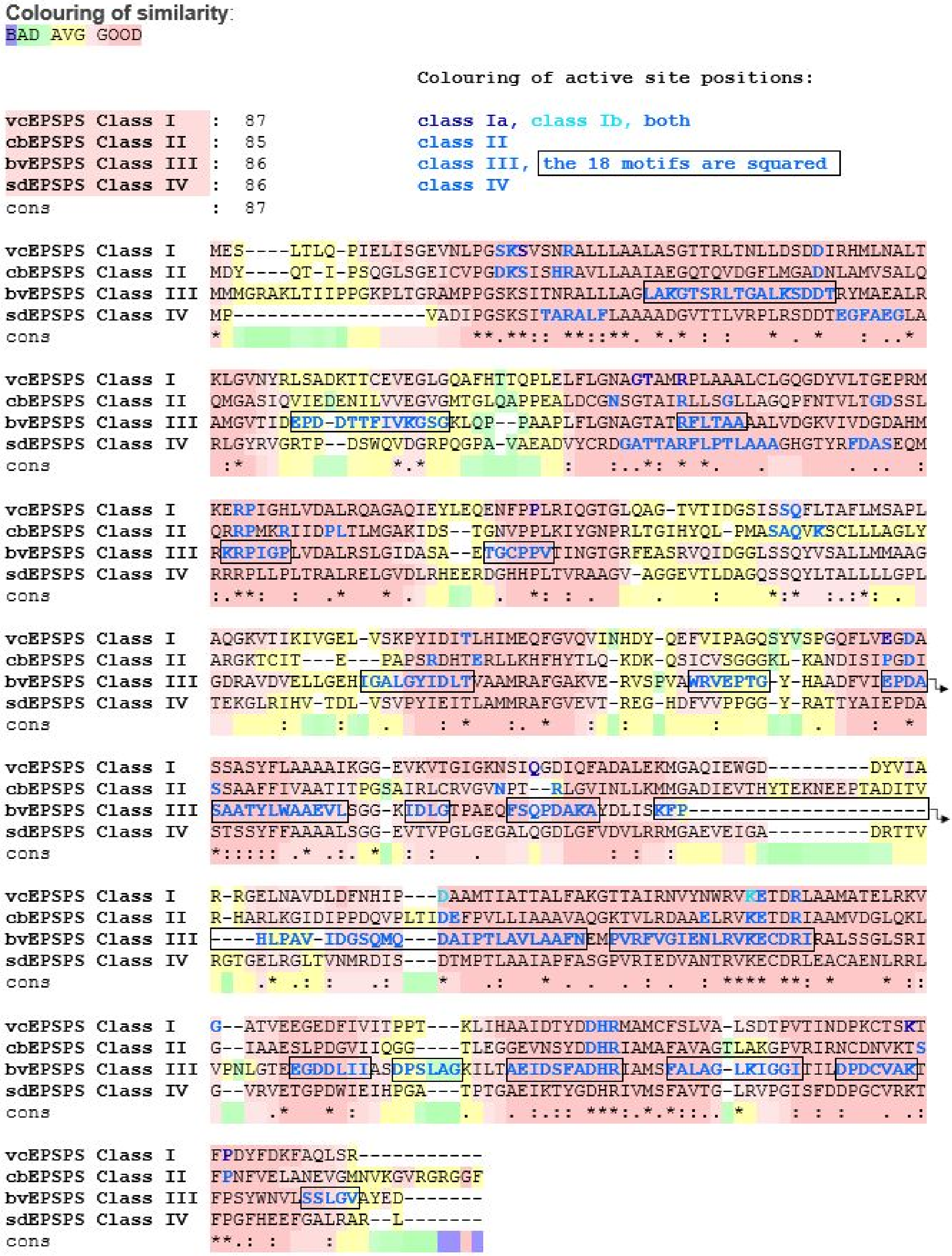
Bryantella formatexigens	1	R	Class II	100%
Clostridium asparagiforme	1	R	Class II	100%
***Clostridium asparagiforme* DSM 15981**	1	R	Class II	100%
Clostridium bartlettii	2	R	Class II	100%
Clostridium scindens	2	R	Class II	100%
***Clostridium sp* L2-50**	2	1×R 1×U	Class II U	50%
***Clostridium sp* SS2-1**	2	R	Class II	100%
***Clostridium symbiosum* ATCC 14940**	1	R	Class II	100%
Coprococcus comes	8	R	Class II	100%
***Coprococcus comes* ATCC 27758**	1	R	Class II	100%
Coprococcus eutactus	6	R	Class II	100%
Dorea formicigenerans	18	R	Class II	100%
Dorea longicatena	11	R	Class II	100%
***Enterococcus casseliflavus* EC10**	1	R	Class II	100%
***Enterococcus casseliflavus* EC20**	1	R	Class II	100%
Eubacterium hallii/Anaerobutycum hallii	9	R	Class II	100%
***Helicobacter canadensis* MIT 98-5491**	1	R	Class II	100%
***Helicobacter cinaedi* CCUG 18818**	2	R	Class II	100%
***Helicobacter pullorum* MIT 98-5489**	1	R	Class II	100%
***Helicobacter winghamensis* ATCC BAA-430**	1	R	Class II	100%
***Lactobacillus brevis subsp. gravesensis* ATCC 27305**	1	R	Class II	100%
***Lactobacillus buchneri* ATCC 11577**	1	R	Class II	100%
***Lactobacillus hilgardii* ATCC 8290**	1	R	Class II	100%
***Lactobacillus plantarum subsp. plantarum* ATCC 14917**	1	R	Class II	100%
***Lactobacillus plantarum* WCFS1**	1	R	Class II	100%
***Lactobacillus ruminis* ATCC 25644**	2	R	Class II	100%
***Listeria grayi* DSM 20601**	1	R	Class II	100%
Mitsuokella multacida	2	R	Class II	100%
***Mitsuokella multacida* DSM 20544**	1	R	Class II	100%
Ruminococcus gnavus	16	R	Class II	100%
***Ruminococcus gnavus* ATCC 29149**	1	R	Class II	100%
Ruminococcus lactaris	2	R	Class II	100%
***Ruminococcus lactaris* ATCC 29176**	1	R	Class II	100%
Ruminococcus obeum	17	R	Class II	100%
***Ruminococcus obeum* A2-162**	1	R	Class II	100%
Ruminococcus torques	7	6×R 1×U	Class II U	86%

Ruminococcus torques **L2-14**	1	R	Class II	100%
Bacteroides pectinophilus **ATCC 43243**	1	U	U	0%
Butyrivibrio crossotus	2	U	U	0%
Clostridium spiroforme **DSM 1552**	1	U	U	0%
Eubacterium rectale **DSM 17629**	1	U	U	0%
Eubacterium rectale **M104 1**	1	U	U	0%
Eubacterium ventriosum	7	U	U	0%
Fusobacterium sp. **2_1_31**	1	U	U	0%
Fusobacterium sp. **D11**	1	U	U	0%
Fusobacterium sp. **D12**	1	U	U	0%
Proteus penneri **ATCC 35198**	1	U	U	0%
Providencia rettgeri **DSM 1131**	1	U	U	0%
Roseburia intestinalis **L1-82**	1	U	U	0%
Roseburia intestinalis **M50 1**	1	U	U	0%

SUPPLEMENTARY FIGURES



Supplementary figure 1. Scheme of the effect of glyphosate on its target enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Glyphosate inhibits the key enzyme of the shikimate pathway and blocks the production of three essential amino acids (phenylalanine, tyrosine and tryptophan).



```
Colouring of similarity:
BAD AVG GOOD

                                      Colouring of active site positions:

vcEPSPS Class I   :  87               class Ia, class Ib, both
cbEPSPS Class II  :  85               class II
bvEPSPS Class III :  86               class III, the 18 motifs are squared
sdEPSPS Class IV  :  86               class IV
cons              :  87

vcEPSPS Class I   MES----LTLQ-PIELISGEVNLPGSKSVSNRALLLAALASGTTRLTNLLDSDDIRHMLNALT
cbEPSPS Class II  MDY----QT-I-PSQGLSGEICVPGDKSISHRAVLLAAIAEGQTQVDGFLMGADNLAMVSALQ
bvEPSPS Class III MMMGRAKLTIIPPGKPLTGRAMPPGSKSITNRALLLAGLAKGTSRLTGALKSDDTRYMAEALR
sdEPSPS Class IV  MP-----------------VADIPGSKSITARALFLAAADGVTTLVRPLRSDDTEGFAEGLA
cons              *                      **.**:: **::**. *.* : :   * . *   : ..*

vcEPSPS Class I   KLGVNYRLSADKTTCEVEGLGQAFHTTQPLELFLGNAGTAMRPLAAALCLGQGDYVLTGEPRM
cbEPSPS Class II  QMGASIQVIEDENILVVEGVGMTGLQAPPEALDCGNSGTAIRLLSGLLAGQPFNTVLTGDSSL
bvEPSPS Class III AMGVTIDEPD-DTTFIVKGSGKLQP--PAAPLFLGNAGTATRFLTAAAALVDGKVIVDGDAHM
sdEPSPS Class IV  RLGYRVGRTP--DSWQVDGRPQGPA-VAEADVYCRDGATTARFLPTLAAAGHGTYRFDASEQM
cons               :*             *.*            :   :..*: * *.   .       . ..  :

vcEPSPS Class I   KERPIGHLVDALRQAGAQIEYLEQENFPPLRIQGTGLQAG-TVTIDGSISSQFLTAFLMSAPL
cbEPSPS Class II  QRRPMKRIIDPLTLMGAKIDS--TGNVPPLKIYGNPRLTGIHYQL-PMASAQVKSCLLLAGLY
bvEPSPS Class III RKRPIGPLVDALRSLGIDASA--ETGCPPVTINGTGRFEASRVQIDGGLSSQYVSALLMMAAG
sdEPSPS Class IV  RRRPLLPLTRALRELGVDLRHEERDGHHPLTVRAAGV-AGGEVTLDAGQSSQYLTALLLLGPL
cons              :.**:  :  .*   * .       .  *: : .     .    :    *:*  :.:*: .

vcEPSPS Class I   AQGKVTIKIVGEL-VSKPYIDITLHIMEQFGVQVINHDY-QEFVIPAGQSYVSPGQFLVEGDA
cbEPSPS Class II  ARGKTCIT---E---PAPSRDHTERLLKHFHYTLQ-KDK-QSICVSGGGKL-KANDISIPGDI
bvEPSPS Class III GDRAVDVELLGEHIGALGYIDLTVAAMRAFGAKVE-RVSPVAWRVEPTG-Y-HAADFVIEPDA↳
sdEPSPS Class IV  TEKGLRIHV-TDL-VSVPYIEITLAMMRAFGVEVT-REG-HDFVVPPGG-Y-RATTYAIEPDA
cons                    :    :   .    : *   :. *   :  :       :        .    :  *

vcEPSPS Class I   SSASYFLAAAAIKGG-EVKVTGIGKNSIQGDIQFADALEKMGAQIEWGD---------DYVIA
cbEPSPS Class II  SSAAFFIVAATITPGSAIRLCRVGVNPT--RLGVINLLKMMGADIEVTHYTEKNEEPTADITV
bvEPSPS Class III SAATYLWAAEVLSGG-KIDLGTPAEQFSQPDAKAYDLISKFP---------------------↳
sdEPSPS Class IV  STSSYFFAAAALSGG-EVTVPGLGEGALQGDLGFVDVLRRMGAEVEIGA---------DRTTV
cons              *::::: .* .:. *  : :   .           : :  :

vcEPSPS Class I   R-RGELNAVDLDFNHIP---DAAMTIATTALFAKGTTAIRNVYNWRVKETDRLAAMATELRKV
cbEPSPS Class II  R-HARLKGIDIPPDQVPLTIDEFPVLLIAAAVAQGKTVLRDAAELRVKETDRIAAMVDGLQKL
bvEPSPS Class III ----HLPAV-IDGSQMQ---DAIPTLAVLAAFNEMPVRFVGIENLRVKECDRIRALSSGLSRI
sdEPSPS Class IV  RGTGELRGLTVNMRDIS---DTMPTLAAIAPFASGPVRIEDVANTRVKECDRLEACAENLRRL
cons                  .* .: :   .:    *   .:   * . .  . : .  : **** **: *    * ::

vcEPSPS Class I   G--ATVEEGEDFIVITPPT---KLIHAAIDTYDDHRMAMCFSLVA-LSDTPVTINDPKCTSKT
cbEPSPS Class II  G--IAAESLPDGVIIQGG----TLEGGEVNSYDDHRIAMAFAVAGTLAKGPVRIRNCDNVKTS
bvEPSPS Class III VPNLGTEEGDDLIIASDPSLAGKILTAEIDSFADHRIAMSFALAG-LKIGGITILDPDCVAKT
sdEPSPS Class IV  G--VRVETGPDWIEIHPGA---TPTGAEIKTYGDHRIVMSFAVTG-LRVPGISFDDPGCVRKT
cons                   .*   * :         .   . :.:: ***:.*.*::.. *    : : :   . .:

vcEPSPS Class I   FPDYFDKFAQLSR-----------
cbEPSPS Class II  FPNFVELANEVGMNVKGVRGRGGF
bvEPSPS Class III FPSYWNVLSSLGVAYED-------
sdEPSPS Class IV  FPGFHEEFGALRAR--L-------
cons              **.: :    :
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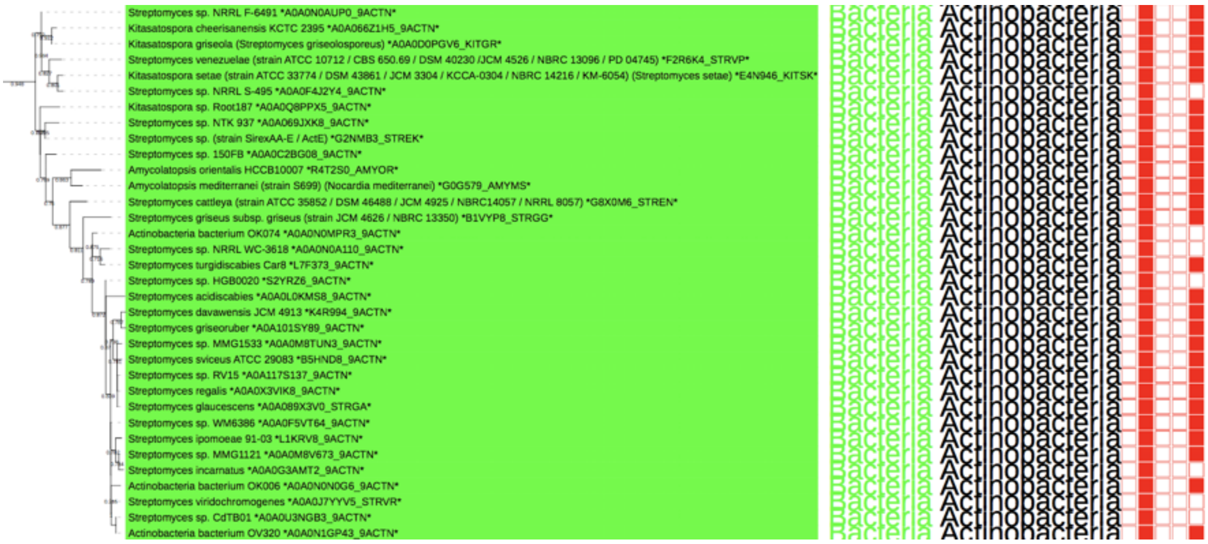
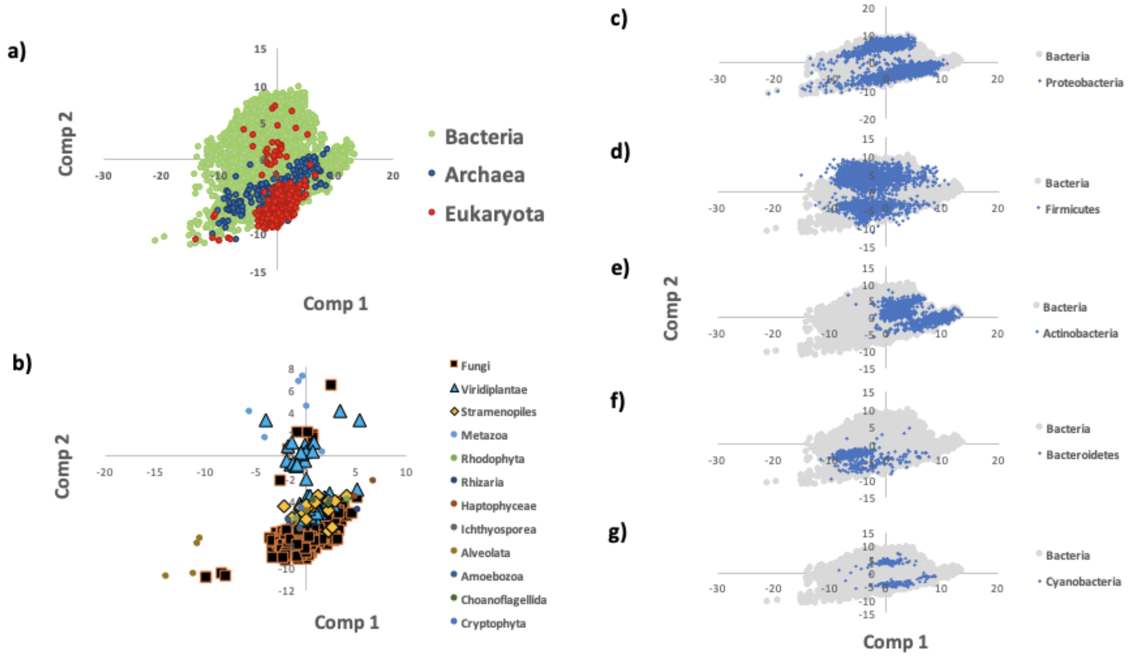
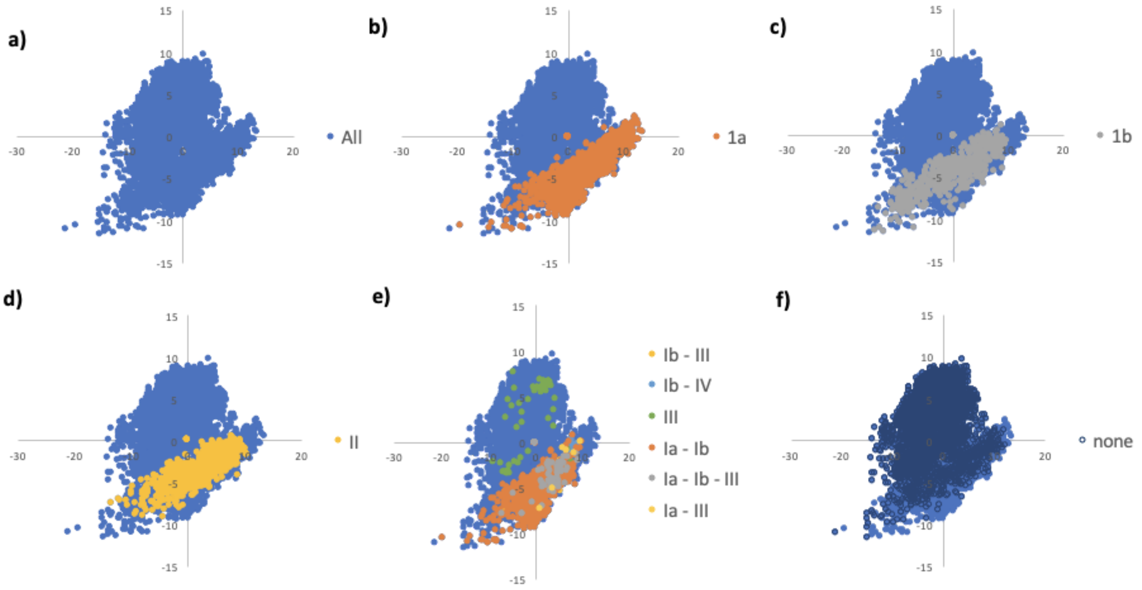
Supplementary figure 2. Multiple sequence alignment made with T-Coffee [3,4] of the four EPSPS reference sequences.



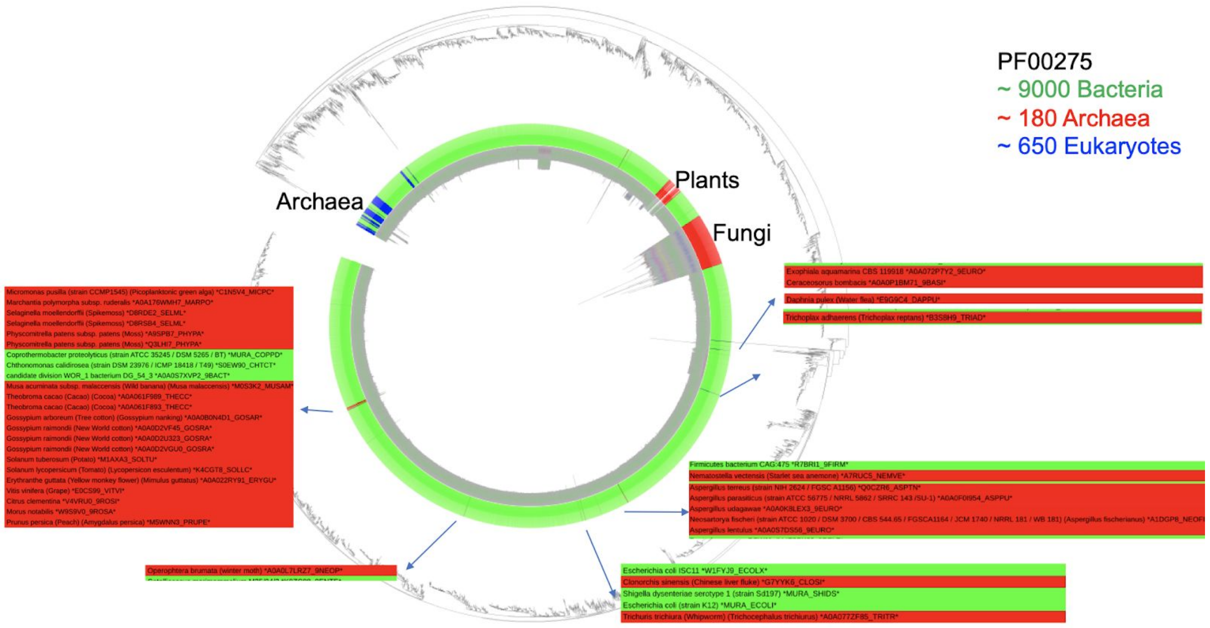
Supplementary figure 3. All EPSPS class IV belong to one single clade of actinobacteria species.



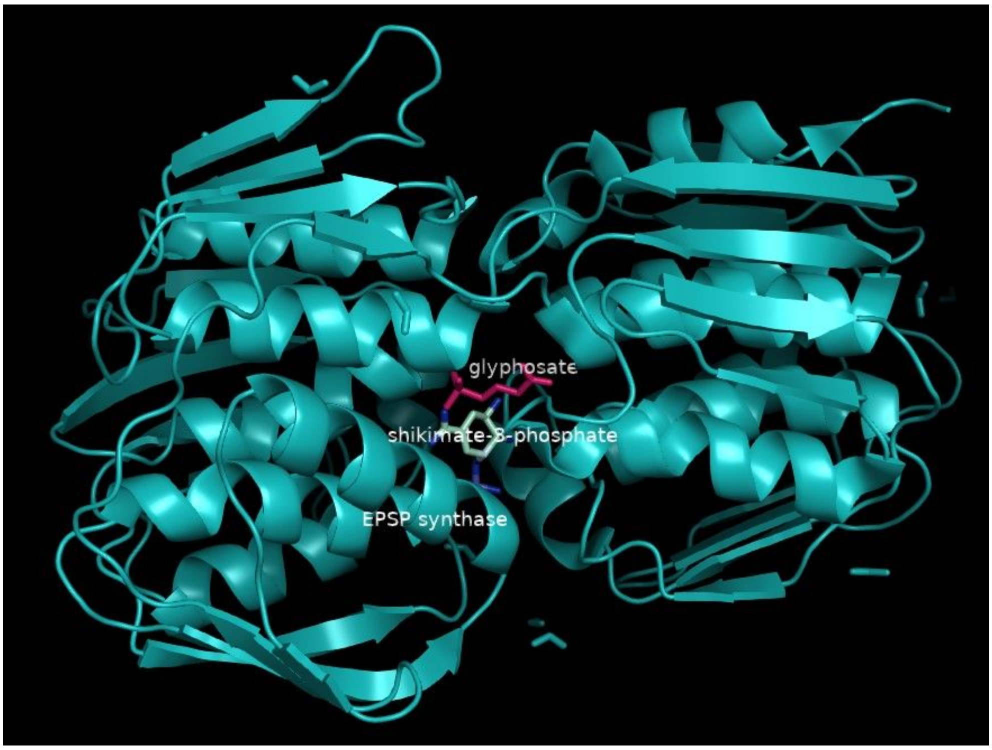
Supplementary figure 4. Principal Components Analysis (PCA) plot of dipeptides by taxonomy
First and second component of the PCA of ~10,000 EPSPS proteins
a) Archaea (blue), bacteria (green) and eukaryotes (red)
b) Eukaryotes c-g) Prokaryotes



Supplementary figure 5. Principal Components Analysis (PCA) plot of dipeptides by EPSPS class
First and second component of the PCA of ~10,000 EPSPS proteins
a) All
b) Class I alpha
c) Class I beta
d) Class II
e) Multiple classes
f) Unclassified



Supplementary figure 6. Eukaryotic species across the species tree (Figure 1a).



Supplementary figure 7. A 3D-structure illustration of the EPSP synthase of *E. coli* (RCSB PDB code: 1G6S) liganded with shikimate-3-phosphate and glyphosate (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC).

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3. Notredame, C., Higgins, D. G. & Heringa, J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J. Mol. Biol.* **302,** 205–217 (2000).
4. Di Tommaso, P. *et al.* T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. *Nucleic Acids Res.* **39,** W13-7 (2011).