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Sucrose phosphorylase from *Alteromonas mediterranea*: structural insight into the regioselective α -glucosylation of (+)-catechin

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Abstract

Sucrose phosphorylases, through transglycosylation reactions, are interesting enzymes that can transfer regioselectively glucose from sucrose, the donor substrate, onto acceptors like flavonoids to form glycoconjugates and hence modulate their solubility and bioactivity. Here, we report for the first time the structure of sucrose phosphorylase from the marine bacteria *Alteromonas mediterranea* (AmSP) and its enzymatic properties. Kinetics of sucrose hydrolysis and transglucosylation capacities on (+)-catechin were investigated. Wild-type enzyme (AmSP-WT) displayed high hydrolytic activity on sucrose and was devoid of transglucosylation activity on (+)-catechin. Two variants, AmSP-Q353F and AmSP-P140D catalysed the regiospecific transglucosylation of (+)-catechin: 89% of a novel compound (+)-catechin-4'-O- α -D-glucopyranoside (CAT-4') for AmSP-P140D and 92% of (+)-catechin-3'-O- α -D-glucopyranoside (CAT-3') for AmSP-Q353F. The compound CAT-4' was fully characterized by NMR and mass spectrometry. An explanation for this difference in regiospecificity was provided at atomic level by molecular docking simulations: AmSP-P140D was found to preferentially bind (+)-catechin in a

23 mode that favours glucosylation on its hydroxyl group in position 4' while the binding mode in
24 AmSP-Q353F favoured glucosylation on its hydroxyl group in position 3'.

25 Keywords: marine microbial enzymes, regioselectivity, biocatalysis, (+)-catechin, sucrose-
26 phosphorylase, *Alteromonas mediterranea*

27 **1. Introduction**

28 Oceans cover more than 70% of Earth's surface and provides a unique environment to marine
29 bacteria (*i.e.* high salinity, high pressure, low temperature and special lighting conditions). For
30 decades, enzymes have been isolated and purified from terrestrial microorganisms, animals and
31 plants. With the advent of biotechnology, there has been a growing interest and demand for
32 enzymes with novel properties and robust biocatalysts. Due to its complexity, the marine
33 environment represents a great opportunity for exploration of new enzymes and molecules [1, 2].
34 Marine enzymes are capable of being active under extreme conditions, which provide
35 competitiveness and efficiency to different industrial processes [3, 4]. Among those, sucrose-
36 phosphorylases (SPs) from the Glycoside Hydrolase family 13 subfamily 18 (GH13_18, EC 2.4.1.7)
37 attract biotechnological interest as biocatalysts. Requiring only a cheap and abundant donor, SPs
38 can perform transglucosylation reaction by transferring glucose from sucrose to an acceptor to
39 yield α -glucosylated products with a retaining mechanism *via* a β -glucosyl-enzyme intermediate.
40 Particularly, *Bifidobacterium adolescentis* SP (*BaSP*-WT) and its mutants have been studied for
41 biocatalytic synthesis of rare disaccharides [5, 6] and α -glucosylation of polyphenols [7, 8, 9, 10].
42 To date, the only documented structures of SP in the literature are from *Bifidobacterium*
43 *adolescentis* [11]. Other SPs, from *Leuconostoc mesenteroides*, *Streptococcus mutans*,
44 *Lactobacillus acidophilus* and *Thermoanaerobacterium thermosaccharolyticum*, have also been
45 studied for the glucosylation of phenolic compounds [12, 13, 14]. Glucosylation of this type of
46 molecules increases their solubility in water and their bioavailability in health, nutraceuticals and

47 cosmetics applications [15, 16]. Controlling the regioselectivity of this glucosylation is also at stake
48 for the synthesis of new compounds. We recently documented the activity of two variants of
49 *BaSP*-WT with respect to their ability to transfer regioselectively a glucose moiety onto (+)-
50 catechin as an acceptor substrate [10]. *BaSP*-Q345F and *BaSP*-P134D/Q345F glucosylated (+)-
51 catechin on hydroxyl groups in position 3' (OH-3') and 5 (OH-5) with obtainment of three
52 glucosylated regioisomers: (+)-catechin-3'-O- α -D-glucopyranoside (CAT-3'), (+)-catechin-5-O- α -D-
53 glucopyranoside (CAT-5) and (+)-catechin-3',5-O- α -D-diglucopyranoside (CAT-3',5), with a ratio of
54 51:25:24 for *BaSP*-Q345F and 82:9:9 for *BaSP*-P134D/Q345F.

55 *Alteromonas mediterranea*, also known as *Alteromonas macleodii* "Deep Ecotype" or AltDE, is an
56 aerobic Gram-negative and mesophilic marine bacterium from the genus of *Proteobacteria* which
57 was first isolated at a depth of 1000 m in the Eastern Mediterranean Sea in 2005 [17, 18, 19]. Wild
58 type form of *AmSP* (*AmSP*-WT) shares 52% of global sequence identity with *BaSP*-WT. Sequence
59 alignments revealed that both enzymes possess highly conserved regions corresponding to the
60 loop A (*BaSP*-WT: ³³⁶AAASNLDLYQ³⁴⁵, *AmSP*-WT: ³⁴⁴AAASNLDLYQ³⁵³) and loop B (*BaSP*-WT:
61 ¹³²YRPRP¹³⁶, *AmSP*-WT: ¹³⁸FRPRP¹⁴²) of the catalytic site [20]. In a preliminary screening using
62 homology modelling and molecular docking, we identified that the catalytic cavity of the glucosyl-
63 intermediate of *AmSP*-WT could potentially host a polyphenolic acceptor compound like (+)-
64 catechin. Towards this end, the crystallographic structure of *AmSP*-WT was for the first time
65 recently determined [Goux *et al.*, in preparation]. In the present work, we characterized *AmSP*-WT
66 from a structural and functional perspective, and further analysed its structural and kinetic
67 features. We also investigated the enzymatic properties of two variants of the enzyme towards
68 their propensity to catalyse the regioselective transglucosylation of (+)-catechin. P140D and Q353F
69 mutations, homologous to mutations P134D and Q345F of *BaSP*-WT, displayed a single transfer
70 reaction product for each enzyme: (+)-catechin-4'-O- α -D-glucopyranoside (CAT-4') for *AmSP*-
71 P140D and CAT-3' for *AmSP*-Q353F. To explain the striking enzymatic activities of those variants,

72 we provide in-depth structural insights by docking simulations and modelling. Our results
73 interestingly broaden the available chemo-enzymatic synthetic tools for the efficient regioselective
74 α -glucosylation of polyphenols.

75 **2. Materials and methods**

76 2.1. *Chemicals*

77 (+)-catechin was purchased from Extrasynthèse and PCR primers from Eurofins. All other chemicals
78 were purchased from Sigma-Aldrich or VWR.

79 2.2. *Vector construction and proteins*

80 AmSP-WT and its variants were expressed as C-terminally hexahistidine-tagged proteins, allowing
81 affinity purification by standard protocols. AmSP-WT gene (UniProt: S5AE64_9ALTE) was ordered
82 from Genscript already cloned in a pET28b vector. Variants AmSP-P140D, AmSP-Q353F and AmSP-
83 P140D/Q353F were obtained by site-directed mutagenesis using primers 1 and 2 for P140D
84 mutation, and primers 3 and 4 for Q353F mutation (Table S1). *E. coli* BL21(DE3) competent cells
85 (Novagen) were transformed and clones were selected using LB-agar medium supplemented with
86 25 μ g/mL kanamycin and confirmed by Sanger sequencing (Eurofins Genomics). Proteins were
87 produced, purified and characterized as previously described [10].

88 2.3. *Kinetics of sucrose hydrolysis*

89 All assays were performed in triplicate in 50 mM MOPS-NaOH at pH 8.0 in PCR strip tubes (Axygen)
90 in a total volume of 100 μ L at 25°C. Sucrose hydrolysis activities of AmSP WT (3 μ M) and variants
91 (10 μ M) were obtained in the presence of sucrose as donor substrate ranging from 1 mM to 50
92 mM. At 0 and 40 min, 50 μ L of reaction volume were sampled and inactivated at 95°C for 5 min.
93 Glucose released due to sucrose hydrolysis was quantified by spectrophotometry thanks to the

94 enzymatic coupled assay using glucose oxidase and peroxidase (GOD-POD), and 2,2'-azino-bis(3-
95 ethylbenzothiazoline-6-sulfonic acid (ABTS) as chromogenic peroxidase substrate. In a 96-well
96 plate, a volume of 25 μ L of each sample were added to 200 μ L of ABTS solution [5 mg/mL ABTS, 5
97 mg/mL glucose oxidase, 5 mg/mL peroxidase, 30 mM citrate buffer-NaOH pH 6.0]. Plates were
98 incubated for 10 min at 25°C inside a Labsystems integrated EIA Management System and
99 absorbance was measured at 405 nm. Kinetic data were fitted to Michaelis-Menten non-linear
100 model and Hanes-Woolf linear model using respectively *nlm* and *lm* functions implemented in R to
101 estimate the kinetic parameters (Figure S3 and S4, Table 1).

102 2.4. Kinetics of the transglucosylation

103 Reactions were carried out in 50 mM MOPS-NaOH solution at pH 8.0 in a total volume of 1 mL.
104 Reaction mixture containing 10 mM (+)-catechin in DMSO (1 eq., 100 μ L), 20% DMSO (100 μ L,
105 (v/v)), 80 mM sucrose in H₂O (8 eq., 100 μ L) was incubated with a final concentration of 10 μ M of
106 purified enzyme at 25°C under slight agitation. Enzymatic synthesis was monitored by thin layer
107 chromatography (TLC) for 24h. TLC plates were developed in solution composed of ethyl
108 acetate/methanol/cyclohexane/water (7:1.5:1:1, v/v/v/v) with 0.1% formic acid (v/v). Products
109 were visualized using a UV lamp at 254 nm and revealed with vanillin-sulphuric acid reagent. After
110 centrifugation (ThermoScientific, Heraeus Pico17 Centrifuge, rotor 75003424, 20 min, 12 000g,
111 20°C), supernatant of the enzymatic reaction medium was analysed by analytical HPLC at 280 nm
112 on a C-18 column (Interchim, 5 μ m, 250 x 4.6 mm, US5C18HQ-250/046) with an isocratic flow of
113 80% H₂O, HCOOH 0.1% (v/v) and 20% MeOH, HCOOH 0.1% (v/v) for 20 min. Compound
114 concentrations were calculated from the area under the curves obtained by analytical HPLC on a
115 C-18 column. The assay was performed in triplicate.

116 2.5. *Purification and analysis of glucosylated (+)-catechin*

117 After 24h of incubation at 25°C under slight agitation, reaction media was centrifuged (12 000g, 20
118 min) and supernatant was purified by HPLC at 280 nm on a C-18 column (Interchim, 5 µm, 250 x
119 21.2 mm, US5C18HQ-250/212) with a gradient system (solvent A: H₂O HCOOH 0.1%; solvent B:
120 MeOH, HCOOH 0.1%; t₀ min = 70/30, t₁₀ min = 70/30, t₇₀ min = 10/90). Products were identified
121 by mass spectroscopy (H⁻ mode, Waters, UPLC-MS2 high resolution) and NMR ¹H and ¹³C in DMSO-
122 d₆ (Bruker, Pulse 400 MHz RS2D, 256 scans). Chemical shifts are quoted in parts per million (ppm)
123 relative to the residual solvent peak. Coupling constant J are quoted in Hz. Multiplicities are
124 indicated as d (doublet), t (triplet), m (multiplet). NMR peak assignments were confirmed using 2D
125 ¹H correlated spectroscopy (COSY), 2D ¹H nuclear Overhauser effect spectroscopy (NOESY) and 2D
126 ¹H-¹³C heteronuclear single quantum coherence (HSQC). Complete characterization of CAT-4' is
127 provided in the ESI, for CAT-3', CAT-5 and CAT-3',5, please see [10].

128

129 2.6. *Docking analysis of binding mode of (+)-catechin in the catalytic pocket*

130 All docking experiments were performed with AutoDock Vina using, for each variant of the
131 enzyme, 50 models of its glucosyl-intermediate form built using Rosetta suite of software [22] and
132 12 (+)-catechin conformers (see Electronic Supplementary Information for details of the
133 modelling). Docking perimeter was limited to the residues of the active site of the enzyme. Each of
134 the 12 conformers of (+)-catechin were docked on every conformer of the two variants of AmSP.
135 This amounts to a total of 600 (50x12) docking experiments for each variant of the enzyme. Only
136 the productive poses that could lead to a glucosylation of (+)-catechin were selected. To do so,
137 docking poses were filtered using the following distance constraints: distances within 3.0 Å
138 between any oxygen of (+)-catechin and the anomeric carbon atom C1 of the glucosyl moiety were

139 assessed. Docking scores were compiled for these productive poses and compared between the
140 two variants.

141 3. Results

142 3.1. Highly conserved structural features and potential activity of AmSP-WT

143 Through structural comparison of *BaSP*-WT (PDB: 1R7A, 2GDV), *BaSP*-Q345F (PDB: 5C8B), and our
144 recently determined structure of *AmSP*-WT (PDB: 7ZNP), we identified the conserved residues
145 likely involved in the reaction mechanism and potential substrate interactions (Table S24). The -1
146 subsite of SPs, also called donor site, has an optimal topology for binding glucose and is conserved
147 between *BaSP*-WT and *AmSP*-WT (Figure 1). The configuration of the two catalytic residues
148 involved in the reaction mechanism are also almost identical (Figure 1A, in blue): a glutamyl
149 residue acts as a general acid/base catalyst (E243 for *AmSP*-WT and E232 for *BaSP*-WT) and an
150 aspartyl residue performs the nucleophilic attack (D203 for *AmSP*-WT and D192 for *BaSP*-WT). The
151 third member of the catalytic triad (D301 for *AmSP*-WT and D290 for *BaSP*-WT) stabilises the
152 transition state with a strong hydrogen bond and presents also an identical configuration in the
153 catalytic site. The structural elements that were shown to stabilize the glucosyl moiety in *BaSP*-WT
154 by non-polar contacts between a hydrophobic platform (F53/F156 for *BaSP*-WT) and the
155 hydrophobic C3-C4-C5 part of glucose are also conserved in *AmSP*-WT (F56/F167). The acceptor or
156 +1 site of SPs is mainly shaped by two highly dynamic loops (Figure S1), which were shown to
157 adopt different conformations based on the progress of the reaction mechanism: one
158 conformation is the “donor binding mode” or closed conformation and the other is the “acceptor
159 binding mode” or open conformation where an arginyl residue (R135 in *BaSP*-WT) is thought to
160 enable the enzyme to outcompete water as an acceptor through strong electrostatic interactions.
161 When we compare the apoenzyme of *BaSP*-WT (PDB: 1R7A) with our newly obtained apoenzyme

162 of AmSP-WT (PDB: 7ZNP), a striking difference in the positioning of loop A is noticed. The enzyme
163 has already an opened conformation and is in the “acceptor binding mode” with Y352 residue
164 pointing inside the active site (Figure 1, in magenta) and R141 pointing outside (Figure 1, in
165 orange). This open conformation was also observed for BaSP-WT crystallized with the end-product
166 of the reaction after hydrolysis of glucose (PDB: 2GDV, chain B). Moreover, crucial conserved
167 residues involved in binding of both phosphate and fructose, Y196 and H234 for BaSP-WT vs. Y207
168 and H245 for AmSP-WT, are in the same conformational positions thus allowing sucrose
169 phosphorylase activity (Figure 1B, in cyan)[21]. In BaSP-WT, Y132 is located at the entrance of the
170 active site and contributes to sucrose specificity thanks to hydrophobic interactions with Y196 and
171 F206. In AmSP-WT, the aromatic structure is conserved with the replacement of the tyrosinyl
172 moiety by a phenylalanyl residue in position 138 (Figure 1B, in orange).

173 3.2. Determination of the apparent kinetic parameters for sucrose hydrolysis

174 C-terminally hexahistidine-tagged AmSP-WT and variants were produced in BL21(DE3) and purified
175 by Ni-NTA immobilized metal affinity chromatography for further characterization. Interestingly,
176 the P140D and Q353F mutations did not alter the enzyme stability, as evidenced by unchanged
177 melting temperature (T_m) of 43°C (Figure S2, Table S3). The apparent kinetic parameters for
178 sucrose hydrolysis at 25°C were determined (Table 1). Globally, AmSP-WT has a higher apparent
179 affinity towards sucrose (lower K_m value) and higher turn-over number (k_{cat}) than the studied
180 variants. Lower activity for sucrose hydrolysis could be indicative of potential increased capacity in
181 transglucosylation. For AmSP-WT, DMSO did not change significantly the K_m and k_{cat} values.
182 Interestingly, for the P140D and Q353F mono-variants, the presence of DMSO 20% increased the
183 k_{cat} two-fold and its specificity constant (k_{cat}/K_m) about 4-fold with respect to absence of co-
184 solvent. With respect to the wild-type enzyme, in presence of DMSO, AmSP-P140D displayed a
185 similar affinity for sucrose similar but a turnover number divided by almost 3-fold, leading to a

186 kinetic efficiency (k_{cat}/K_m) divided by two. In the same conditions, AmSP-Q353F ($K_m = 2$ mM) has
187 a two fold decrease in affinity with respect to AmSP-WT ($K_m = 1$ mM) and a corresponding 19-fold
188 decrease in its turnover number.

189 3.3. (+)-catechin transglucosylation studies

190 We assessed the ability of SPs to transfer a glucose moiety from sucrose to (+)-catechin at 25°C
191 after 24h under agitation (Figure S3). Observed products were purified by preparative HPLC and
192 analysed by NMR and mass spectrometry. AmSP-P140D and AmSP-Q353F catalyse efficiently the
193 synthesis of two different regioisomers of (+)-catechin glucoside: AmSP-P140D glucosylated mostly
194 the hydroxyl groups in position 4' (OH-4') while AmSP-Q353F glucosylated the OH-3' position
195 (Figures S5B and S5C, Table S4). We monitored the synthesis of glucosylated (+)-catechin products
196 during 24h by HPLC to determine conversion yields and proportion of regioisomers (Figure 2, Table
197 2). Interestingly, the main product formed with AmSP-P140D was CAT-4' with a relative proportion
198 of 89% while the main product formed with AmSP-Q353F was CAT-3' with a relative proportion of
199 92%. The corresponding synthetic yields (percentage of (+)-catechin that was converted into these
200 glycosylated products) was 26% and 82% respectively. These results clearly indicate that these
201 two variants are highly regioselective with respect to their transglycosylation activities on (+)-
202 catechin.

203 3.4. Structural insights into the regioselectivity of AmSP-P140D and AmSP-Q353F

204 To further understand the observed regioselectivities, we performed molecular docking
205 simulations on AmSP-Q353F and AmSP-P140D. The preferred orientations of (+)-catechin in the
206 acceptor site of the glucosyl-enzyme intermediate were assessed. Docking results were consistent
207 with the observed experimental regioselectivity. For AmSP-Q353F, binding mode of (+)-catechin
208 suggest preference for transglycosylation in OH3' position (Figures 3 and S7). On the other hand,

209 for *AmSP*-P140D, the binding mode of (+)-catechin suggest preference for transglycosylation in
210 OH-4' position (Figures 3 and S7). For *AmSP*-P140D, (+)-catechin is stabilized in the active +1 site
211 by a network of 4 hydrogen bonds and numerous hydrophobic contacts (Figure 4).

212 4. Discussion

213 In glycochemistry, the fine control of the regioselectivity is the Holy Grail in enzymatic reactions
214 catalysed by glycosyl hydrolase (GHs) such as sucrose phosphorylase. A disadvantage of GHs is
215 their moderate regioselectivity, meaning that a mixture of products is often formed when the
216 acceptor contains more than one hydroxyl group. Previously, for (+)-catechin which consists of five
217 phenolic hydroxyl groups, we generated with *BaSP*-Q345F and *BaSP*-P134D/Q345F a mixture of
218 glucosylated regioisomers: CAT-3', CAT-5 and CAT-3',5 with a ratio of 51:25:24 for *BaSP*-Q345F and
219 82:9:9 for *BaSP*-P134D/Q345F. Another drawback is the relatively low product yields. With the
220 same variants, we obtained a synthetic yield of 34%/15%/9% and 40%/5%/4%, respectively. In the
221 active site of the SPs, the -1 site is rigid to allow a high selectivity on glucose while the +1 site is
222 more flexible and can accept several types of acceptors or leaving groups. The +1 site of *AmSP*-WT,
223 is mainly shaped by two highly labile loops, loop A (³⁴⁴AAASNLDLYQ³⁵³) and loop B (¹³⁸FRPRP¹⁴²),
224 which undergo crucial conformational changes throughout the catalytic cycle suited for binding
225 either fructose or phosphate. Crystal structure of *AmSP*-WT shows a wide access channel capable
226 of accommodating naturally large polyphenolic acceptors. We decided to perform homologous
227 mutations as what was done on *BaSP* to check their impact on regioselectivity in the context of
228 *AmSP*. By site-directed mutagenesis, we hence substituted the residue Q353 in loop A into F353
229 and/or the residue P140 into D140 in Loop B. We obtained three variants: *AmSP*-P140D, *AmSP*-
230 Q353F and *AmSP*-P140D/Q353F. The double variant showed no improvement for the synthesis of
231 CAT-3' with the obtainment of a mixture of products at 25°C (Figure S5D) and was not further
232 characterized.

233

234 By engineering only the residue 353 of the active site, we obtained an enhanced regioselectivity
235 with glucosylation at OH-3' position almost exclusively (Figure S5B). Interestingly, we also noticed
236 that transglucosylation is most favored for this variant compared to hydrolysis in presence of (+)-
237 catechin. Indeed, transglucosylation reaction proceeded to nearly completion within 24h (Figure 2)
238 for AmSP-Q353F while rate of sucrose hydrolysis was very low (Table 1). AmSP-Q353F, as it was
239 shown for BaSP-Q345F and BaSP-P134D/Q345F, preferentially glucosylate the OH-3' position of
240 flavonoids while ignoring the OH-4' position. Docking studies confirmed that the most favoured
241 pose for (+)-catechin in the catalytic +1 site of AmSP-Q353F lead to the formation of CAT-3' (Figure
242 3A and 3C, and Table 2). As seen with BaSP-Q345F, we hypothesized that the introduction of F353
243 as a potential partner for π - π stacking would lead to rearrangements in loop A with a shift of Y352
244 which could stabilize (+)-catechin in the active site by hydrophobic interactions (Figures 4B and
245 S8).

246 Surprisingly, while BaSP-P140D was not active, AmSP-P140D leads to the regioselective formation
247 of CAT-4' (Figure S5C and Table 2). Thus, the regioselectivity of AmSP shifted completely from CAT-
248 3' in Q353F variant to CAT-4' in P140D variant. An explanation enlightened by molecular modelling
249 is the steric hindrance caused by F138/Y207/F217 residues in AmSP-P140D that does not favor
250 orientation of the substrate for CAT-3' synthesis (Figures 3A, 3B and S8). Indeed, we observed that
251 the conformation of the acceptor site drastically changed and seems to allow only an almost linear
252 orientation of all three (+)-catechin rings (Figure S8). Contrarily to AmSP-Q353F, those very strong
253 constraints lead to the regioselective glucosylation of the OH-4' position of the flavonoid with a
254 high proportion (89%). However, as shown in Figure 2, overall yield does not exceed 26% for the
255 CAT-4' transglucosylation product. We hypothesized that it is mainly due to the possible
256 competition between (+)-catechin, sucrose and water into the active site, favoring the sucrose
257 hydrolysis reaction (Figures S3 and S4).

258 5. Conclusion

259 In this study, we provided the first report of the use of variants of sucrose phosphorylase from
260 *Alteromonas mediterranea* for the regioselective transglucosylation of (+)-catechin and the
261 synthesis of a novel compound fully characterized, (+)-catechin-4'-O- α -D-glucopyranoside (CAT-4').
262 AmSP-Q353F and AmSP-P140D are able to synthesize regioselectively compound CAT-3' and CAT-
263 4', with a proportion of 92% and 89%, respectively. With AmSP-P140D, we succeed to switch the
264 regioselectivity from OH-3' to OH-4'-glucosylated (+)-catechin. Mutation P140D changes drastically
265 the conformation of the acceptor site and seems to allow an almost linear alignment of the
266 glucose moiety and of all three (+)-catechin rings allowing selectively the glucosylation of the
267 position OH-4' of this flavonoid. Overall, the results described herein suggest that AmSP-Q353F
268 and AmSP-P140D are suitable for the enzymatic regioselective synthesis of polyphenolic glucosides
269 at high yields and could facilitate the synthesis of *de novo* products in OH-4' position using other
270 phenolic phytochemicals such as quercetin or kaempferol.

271 Supporting information

272 Details about AmSP-WT sequence, primers, melting curves, kinetic parameters analysis,
273 transglucosylation studies, HPLC/MS and NMR spectra are provided in the Electronic
274 Supplementary Information.

275 Author Contributions

276 MG and MD wrote the original draft. MG, MD, CM, BO and JH developed the methodology. MG,
277 MD, CS and EL performed the experimental investigation and the subsequent analysis. DT and FF
278 obtained the crystallographic structure of AmSP-WT. JH and BO performed the molecular and

279 docking simulations and the following analysis. CM obtained the funding, designed and directed
280 the project. All authors discussed the results and contributed to the final manuscript.

281 **Conflicts of interest**

282 There are no conflicts to declare.

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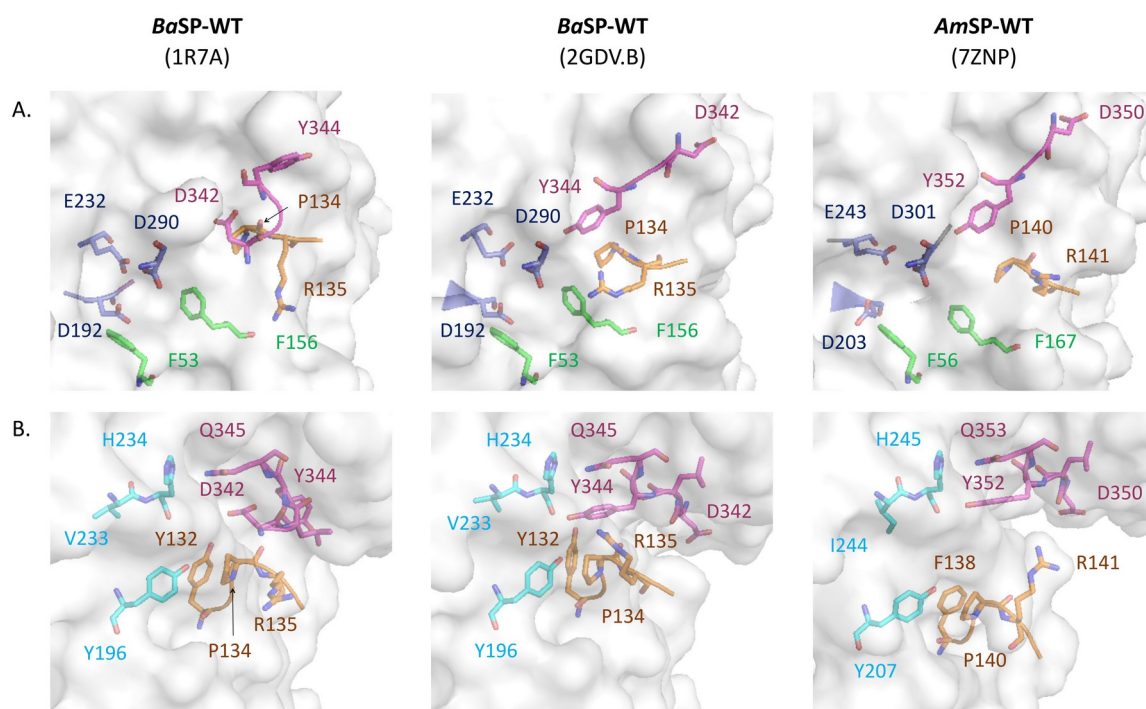
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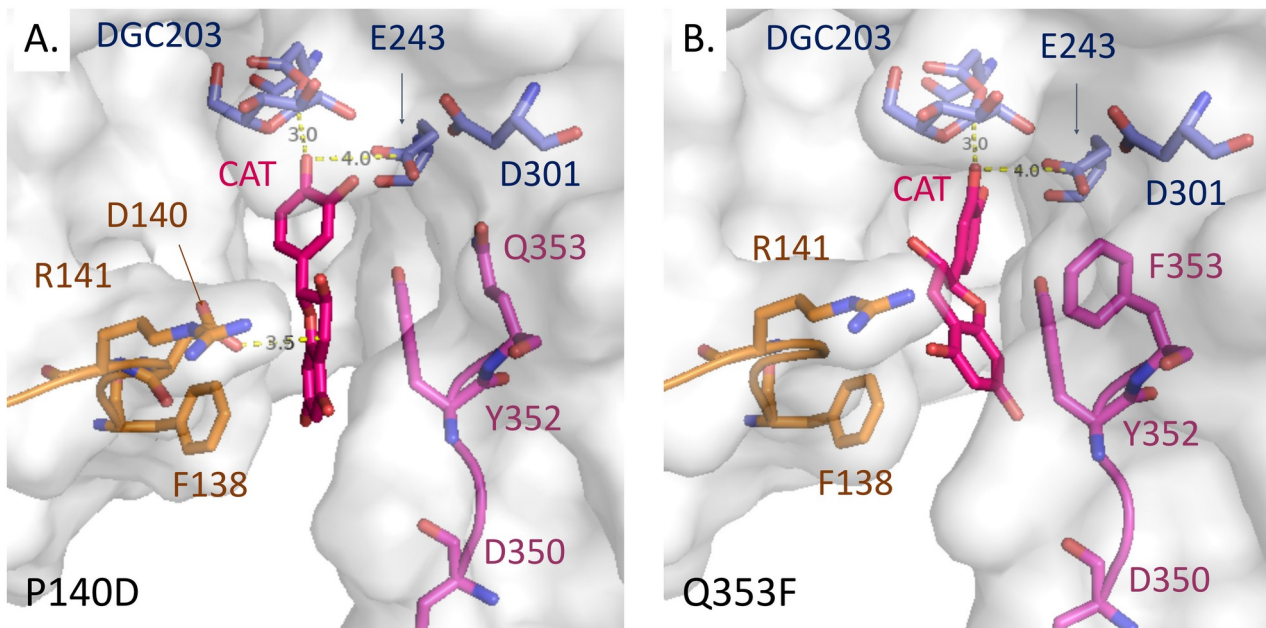


363

364 **Figure 1: Crystallographic structures of *BaSP*-WT and *AmSP*-WT focused on residues involved in**365 **(A) sucrose and (B) fructose binding. (A)** In magenta: Loop A (in sticks for *BaSP*-WT: Y344/D342, in366 sticks for *AmSP*-WT: Y352/D350); orange: Loop B (in sticks for *BaSP*-WT: R135, in sticks for *AmSP*-367 WT: R141); green: hydrophobic platform (in sticks for *BaSP*-WT: F53 and F156, in sticks for *AmSP*-368 WT: F56 and F167); and blue: residues of the catalytic triad (in sticks for *BaSP*-WT:369 D192/E232/D290, in sticks for *AmSP*-WT: D203/E243/D301). **(B)** In magenta: Loop A (in sticks for370 *BaSP*-WT: D342/L343/Y344/Q345, in sticks for *AmSP*-WT: D350/L351/Y352/Q353); orange: Loop B371 (in sticks for *BaSP*-WT: Y132/R133/P134/R135, in sticks for *AmSP*-WT: F138/R139/P140/R141);372 Cyan: residues involved in sucrose phosphorylase activity (in sticks for *BaSP*-WT: Y196/V233/H234,373 in sticks for *AmSP*-WT: Y207/I244/H245).

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377 **Figure 2: Products profile of AmSP-Q353F and AmSP-P140D using (+)-catechin as acceptor.**

378 Compound concentrations were calculated from the area under the curves obtained by analytical

379 HPLC on a C-18 column for 7h and at 24 h of incubation at 25°C of the reaction mixture in a final

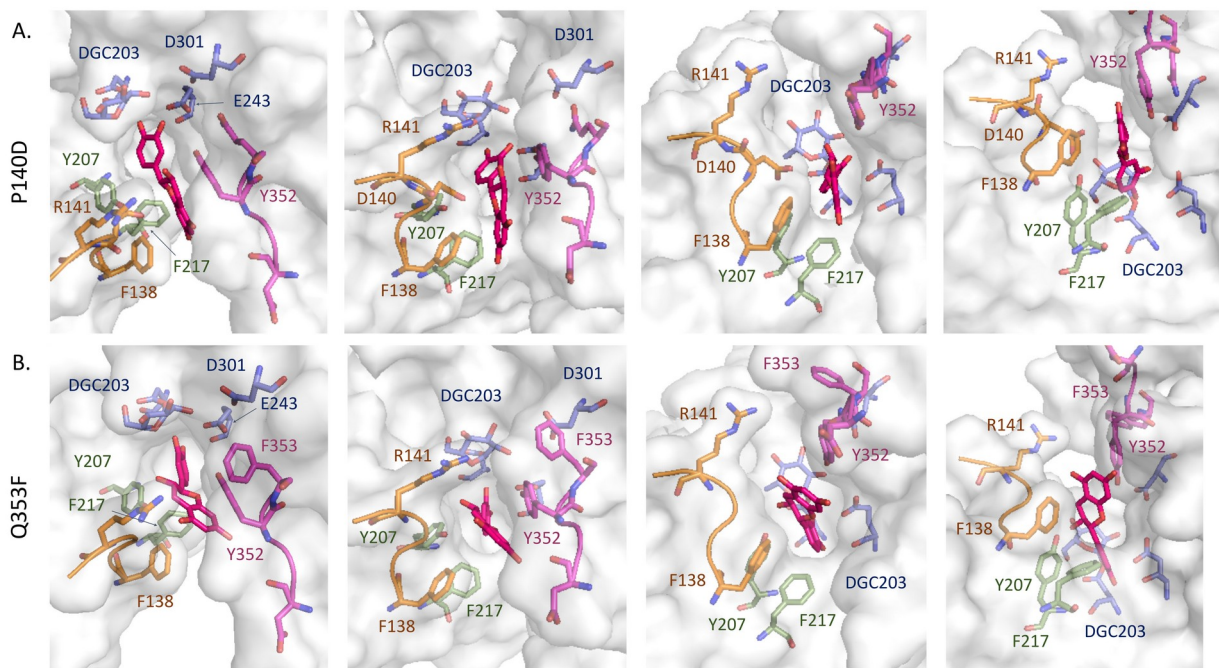
380 volume of 1 mL in MOPS-NaOH 50 mM pH 8.0 with 10 µM of enzyme, 80 mM sucrose and 20%

381 DMSO (v/v) (n=3). Errors correspond to standard deviations. Details of the data can be found in ESI

382 (Table S4).

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385

386 **Figure 3: Comparison of the binding mode of (+)-catechin in AmSP-P140D and AmSP-Q353F. (A)**

387 Best binding mode of (+)-catechin in AmSP-P140D glucosyl-enzyme intermediate. (B) Best binding

388 mode of (+)-catechin in AmSP-Q353F glucosyl-enzyme intermediate. In magenta, loop A with in

389 sticks Y352/D350/Q(F)353 residues; in orange, loop B in orange with in sticks R141/F138 and D140

390 for AmSP-P140D; in blue, residues of the catalytic triad with in sticks DGC203/E243/D301; in pink,

391 (+)-catechin.

392

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400

401 **Table 1: Apparent kinetic parameters for sucrose hydrolysis by AmSP and its variants.**

402 Data were obtained by glucose titration using GOD/POD method of the reaction medium that
 403 reacted for 3h at 25°C and that contained the SP enzyme (10 μ M for variants and 3 μ M for WT) and
 404 1 mM to 50 mM of sucrose, in MOPS-NaOH 50 mM pH 8.0 (n=3). Values are based on linear fit to
 405 the Hanes-Woolf model. Michaelis-Menten and Hanes-Woolf plots are provided in the ESI (Figures
 406 S3 and S4).

	0% DMSO			20% DMSO		
	K_M (mM)	k_{cat} (min ⁻¹)	k_{cat}/K_M	K_M (mM)	k_{cat} (min ⁻¹)	k_{cat}/K_M
<i>AmSP-WT</i>	1.3	56.9	42.8	1.5	68.4	46.9
<i>AmSP-P140D</i>	2.2	14.7	6.7	1.0	24.3	24.1
<i>AmSP-Q353F</i>	7.1	3.0	0.4	2.3	5.6	2.5
<i>AmSP-P140D/Q353F</i>	12.6	2.1	0.2	6.9	1.1	0.2

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408

409 **Table 2: Synthetic yields (A) and relative proportion (B) of (+)-catechin glycosylated products**
 410 **obtained with P140D and Q353F variants of AmSP.** Synthetic yields and relative proportions of
 411 each product are expressed as a percentage of (+)-catechin that was converted into the
 412 corresponding glycosylated products (CAT-4': (+)-catechin-4'-O- α -D-glucopyranoside; CAT-3':
 413 (+)-catechin-3'-O- α -D-glucopyranoside; CAT-5: (+)-catechin-5-O- α -D-glucopyranoside; CAT3',5:
 414 (+)-catechin-3',5-O- α -D-digluco-pyranoside). The relative proportion of each product was calculated
 415 from the area under the curves obtained by analytical HPLC at 24 h with the same conditions than
 416 for Figure S5. Values were obtained from the area under the curves obtained by analytical HPLC of
 417 the reaction mixture containing 10 μ M enzyme, 10 mM (+)-catechin, 80 mM sucrose, 20% DMSO
 418 (v/v) in MOPS 50 mM pH 8.0, and incubated at 25°C for 24 h. Details of the data can be found in
 419 ESI (Table S4, Figure S5).

Product	Synthetic yields				Proportion of each product			
	CAT-4'	CAT-3'	CAT-5	CAT-3',5	CAT-4'	CAT-3'	CAT-5	CAT-3',5
P140D	26%	3%	Traces	Traces	89%	11%	Traces	Traces
Q353F	3%	83%	2%	2%	4%	92%	2%	2%

420