

1 **Supplementary material**

2 **Mechanism-based site-directed mutagenesis to shift the optimum pH of phenylalanine**  
3 **ammonia-lyase from *Rhodotorula glutinis* JN-1**

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1 **Supplementary table**

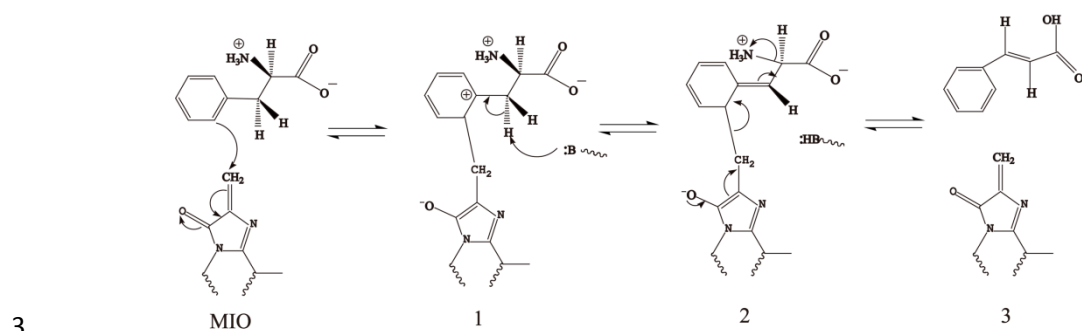
2 **Table S1 Oligonucleotide primers used for site-directed mutagenesis**

Mutant enzyme	Nucleotide sequence (5'→3') <sup>a</sup>
Wt- PAL-up	<b>GGAATTCCAT</b> ATGATGGCCCCCTCCGTCGACTCGATC
Wt- PAL-down	CG <b>GGAATTC</b> CTAGTATGGTCTACGTCCAAAGG
RgPAL-H136F	CAG AAG GCT CTG CTC GAG <u>TTC</u> (CAC) CAA CTC TGC GGT
RgPAL-H136E	CAG AAG GCT CTG CTC GAG <u>GAG</u> (CAC) CAA CTC TGC GGT
RgPAL-Q137L	CAG AAG GCT CTG CTC GAG CAC <u>CTC</u> (CAA) CTC TGC GGT
RgPAL-Q137E	CAG AAG GCT CTG CTC GAG CAC <u>GTG</u> (CAA) CTC TGC GGT
RgPAL-H136K	CAG AAG GCT CTG CTC GAG <u>AAG</u> (CAC) CAA CTC TGC GGT
RgPAL-Q137K	CAG AAG GCT CTG CTC GAG CAC <u>AAG</u> (CAA) CTC TGC GGT
RgPAL-Δ136H	CAG AAG GCT CTG CTC GAG (CAC) CAA CTC TGC GGT
RgPAL-Δ137Q	CAG AAG GCT CTG CTC GAG CAC (CAA) CTC TGC GGT

- 3 <sup>a</sup> Nucleotides underlined correspond to the codon chosen for mutation. Nucleotides in parentheses
- 4 replace the underlined nucleotides. Bold letters denote the *NdeI* and *EcoRI* restriction sites in PAL-up
- 5 and PAL-down, respectively. Δ indicated the truncation.

1 **Supplementary figures**

2 **Fig. S1 The Friedel-Crafts-type mechanism of *Rg*PAL**



4 The mechanism for PAL was consulted from the reports of Calabrese (2004), Poppe (2005) and  
5 Strom (2012). In the Friedel-Crafts-type reaction, the MIO attack the phenyl ring of the substrate to  
6 form carbocation **1** which would stabilize intermediate **2** formed by removal of the substrate's C3  
7 hydrogen; **3**, Collapse of the system to product occurs with the elimination of NH<sub>3</sub> and the release of  
8 cinnamate from the MIO.