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Supporting information for article:

Structure of the plant growth-promoting factor YxaL from the rhizobacterium *Bacillus velezensis* and its application to protein engineering

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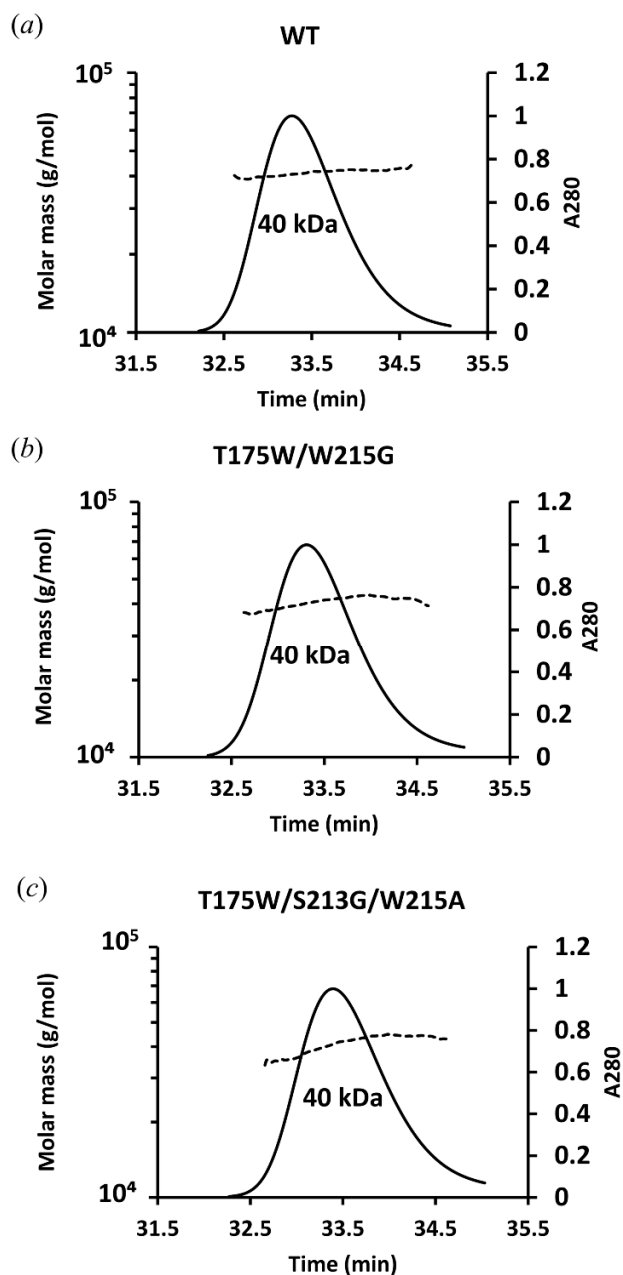


Figure S1 SEC-MALS results for the wild-type and mutant YxaL proteins. SEC-MALS results for the wild-type (a), T175W/W215G mutant (b), and T175W/S213G/W215A mutant (c) YxaL proteins. The SEC profiles in the solid lines were drawn with the absorbance at 280 nm (A_{280}) on the right axis, and the molecular sizes calculated by MALS are in dotted lines on the left axis with estimated values.

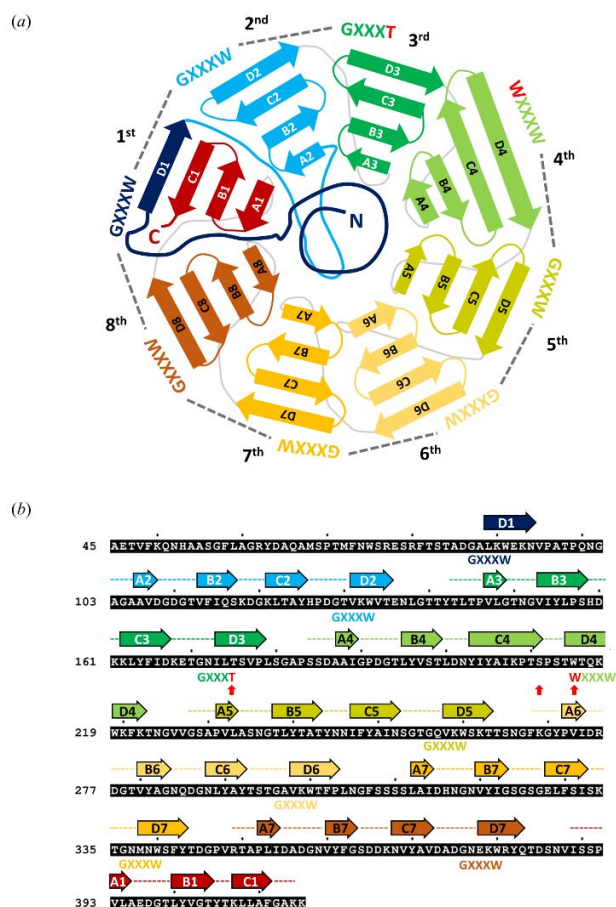


Figure S2 The topological diagram of the wild-type YxaL protein. (a) Topological diagram of the structure of the wild-type YxaL protein from the top view. β -strands are labeled in alphabet and number (A1-D8). The N-terminus (N), C-terminus (C), and order of blades are annotated. The sequences of the conserved GXXXW motifs and their modified motifs are shown. (b) Sequences of YxaL aligned with the blades. β -Strands are labeled in alphabet and number (A1-D8). The sequences of the conserved GXXXW motifs and the variations at the GXXXW motif are shown. Red arrows indicate engineered amino acid positions. Amino acids colored in red indicates variations at the GXXXW motif.

| | | | | | | | | | | | |
|-------------------|----------|---|------------|---|---|----------|----------|-----|---|---|----------|
| Motif 1 (81-91) | F | T | S | T | A | D | <u>G</u> | A | L | K | <u>W</u> |
| Motif 2 (123-133) | T | A | Y | H | P | D | <u>G</u> | T | V | K | <u>W</u> |
| Motif 3 (165-175) | F | I | <u>D</u> | K | E | <u>T</u> | <u>G</u> | N | I | L | <u>T</u> |
| Motif 4 (209-219) | P | T | S | P | S | <u>T</u> | <u>W</u> | T | Q | K | <u>W</u> |
| Motif 5 (250-260) | <u>A</u> | I | <u>N</u> | S | G | <u>T</u> | <u>G</u> | Q | V | K | <u>W</u> |
| Motif 6 (290-300) | Y | A | Y | T | S | <u>T</u> | <u>G</u> | A | V | K | <u>W</u> |
| Motif 7 (330-340) | F | S | I | S | K | <u>T</u> | <u>G</u> | N | M | N | <u>W</u> |
| Motif 8 (370-380) | Y | A | V | D | A | D | <u>G</u> | N | E | K | <u>W</u> |
| MDH PQQ motif | <u>A</u> | X | <u>D/N</u> | X | X | T | <u>G</u> | D/E | X | X | <u>W</u> |
| GDH/ADH PQQ motif | <u>A</u> | X | <u>D/N</u> | X | X | T | <u>G</u> | K | X | X | <u>W</u> |

Figure S3 The PQQ motifs in each blade of YxaL, compared to the PQQ dehydrogenase family. Amino acid sequences of the tryptophan docking motifs of YxaL are presented based on the crystal structure. The consensus PQQ motifs of the typical methanol dehydrogenase (MDH), glucose dehydrogenase (GDH), and alcohol dehydrogenase (ADH) family are in the bottom lines. Amino acids with underline are identical to consensus sequences. Gly and Trp residues in conserved GXXXW motif are colored in blue. The amino acids in red indicates the variations at the GXXXW motif in the third and fourth blades.

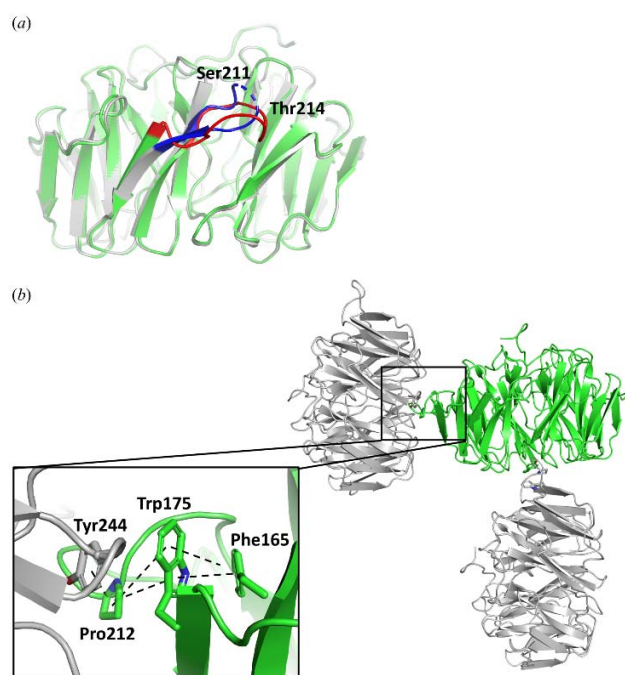


Figure S4 Crystal structure of the T175W/W215G mutant YxaL protein. (a) The structures of the asymmetric unit of the T175W/W215G mutant YxaL consisting of two protomers (chains A and B). The chain A (green) were aligned with the chain B (gray) in the ribbon representations. The loops connecting β -strand C4 to β -strand D4 are colored red and blue, respectively, in the chains A and B. In the chain B, residues 211-214, which were not well defined in the crystal structure, are shown as dotted lines. (b) The structures of chain A of the T175W/W215G mutant YxaL (green) with symmetry mates (gray) shown with ribbon representations. The boxed region indicating the crystal contacts is enlarged in the left panel. Each residue related to the interactions between protomers is shown in the stick model. The π interactions between Phe165, the mutated Trp175, and Pro213 of chain A and Tyr244 of the symmetry mate are shown in dotted lines.

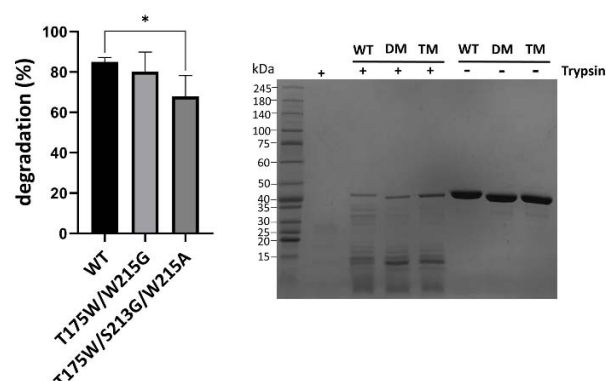


Figure S5 Comparison of the protease resistance between the wild-type and engineered YxaL proteins. Tryptic digestion of the wild-type (WT), T175W/W215G mutant, and T175W/S213G/W215A mutant YxaL proteins. The degradation percentages of the wild-type and mutant YxaL proteins are represented in bar graphs in the left panel, showing the means \pm SD from three individual experiments. Individual groups of the wild-type and mutant YxaL proteins were compared using the *t*-test. *, $P \leq 0.05$. The SDS polyacrylamide gel image of the wild-type (WT), T175W/W215G mutant (DM), and T175W/S213G/W215A mutant (TM) YxaL proteins is shown in the right panel. The presence or absence of trypsin is shown on the image.

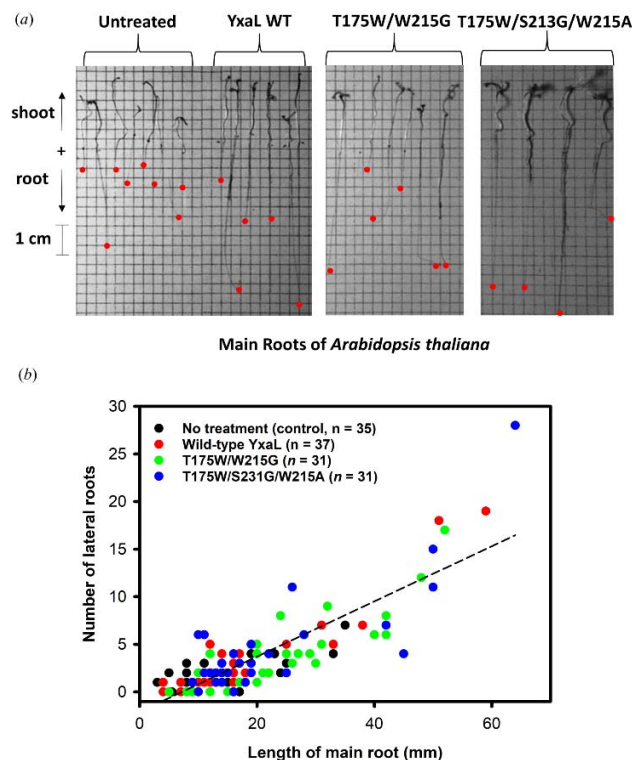


Figure S6 The supporting results for Fig. 5. (a) Morphologies of 2-week-old *A. thaliana* seedlings planted after 2 h treatment with or without addition of 1 $\mu\text{L}/\text{mL}$ each of wild-type, T175W/W215G, and T175W/S213G/W215A mutant YxaL proteins to soaking solutions. The '+' sign indicates the boundary between the shoot and the root regions. Red dots indicate the end of the roots. The interval of three grids is 1 cm. (b) Correlation between the main root length and the lateral root number of 2-week-old *A. thaliana* seedlings, untreated and treated with wild-type and mutant YxaL proteins. The value of Pearson's correlation coefficient (r) for all seedlings is 0.853.

Table S1 The nucleotide sequences of the primers.

| | |
|---------------|---|
| T175W_F | 5'-AGAAACCGGAAACATTTTAT <u>TGG</u> TCGGTTCGCTGAGCGGAGC |
| T175W_R | 5'-TTGTCGATGAAATACAGTTTTTTATCG |
| W215G_F | 5'-ACCGACGTCGCCTTCGACAG <u>GG</u> ACGCAGAAGTGGAAGT |
| W215G_R | 5'-TTAATCGCATAGATATAGTTATCC |
| S213G-W215A_F | 5'-ACCGACGTCGCCT <u>GGG</u> ACAG <u>CG</u> ACGCAGAAGTGGAAGT |