

Supplementary Tables

Table S1. Total and specific activities during purification of His₆-YwqN

Cell fraction	^a Total activity (nM of methyl red reduced/min)	^a Specific Activity (mM of methyl red reduced/min/μg of protein)
Soluble cell-free extract	362.6 ± 26.7	16.9 ± 1.2
Flow through	104.5 ± 41.7	7.7 ± 2.4
Purified protein	996 ± 71	153.4 ± 11.2

^aAverage of 3 experiments ± SD

Table S2. Specific activities of *Bs*YwqN over distinct substrates.

Substrate	^a Specific Activity (μM of substrate reduced/min/μg of protein)
Methyl red	1.75 ± 0.13
Methyl orange	1.54 ± 0.27
Disperse orange	0.81 ± 0.1
^b Menadione	141.2 ± 0.36
^b Naphtoquinone	155.9 ± 16
^c Cr (VI)	0.21 ± 0.05

^aAverage of three independent experiments ± SD. ^bConcentration (μM) of NADPH reduced. ^cDetermined spectrophotometrically at 540 nm, using 1,5-diphenylcarbazide (DPC) as the complexing agent, as previously described (7).

Supplementary Figures.

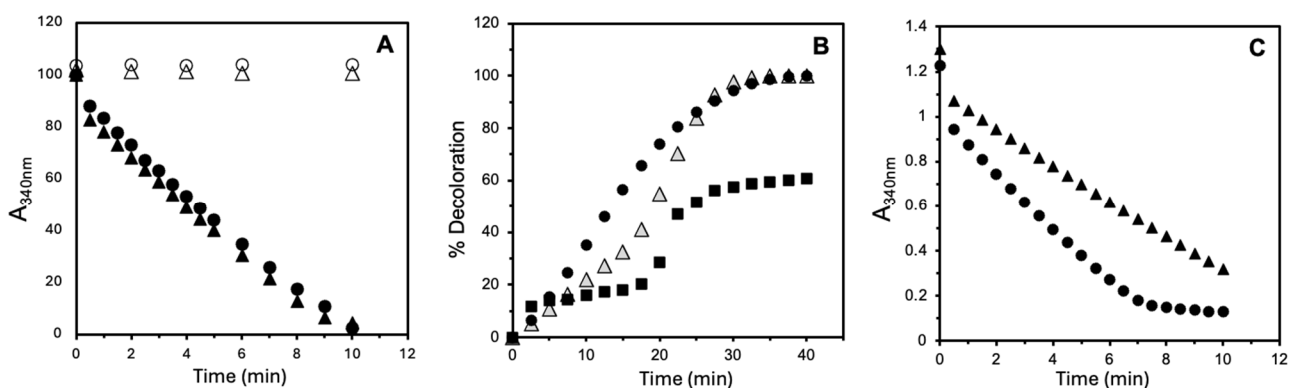


Figure S1. (A) NAD(P)H-FMN reductase activity, (B) Azoreductase activity, (C) Quinone Reductase activity of YwqN. A) 100 mM NADH (circles) or NADPH (triangles) were mixed with (filled symbols) or not (open symbols), 0.5 μ M enzyme at pH 7.5 and room temperature and recorded at 340 nm for 10 min. B) 60 μ M of methyl red (circles), methyl orange (triangles) or disperse orange (squares) were mixed with 0.5 μ M of His₆-YwqN and 1mM of NADPH at pH 7.5 and room temperature and recorded at 430, 460 and 445nm respectively for 40 min. C) 50 μ M of menadione (circles) or naphthoquinone (triangles) were mixed with 0.5 mM of His₆-YwqN and 300 mM of NADPH at pH 7.5 and room temperature and recorded at 340 nm for 10 min.

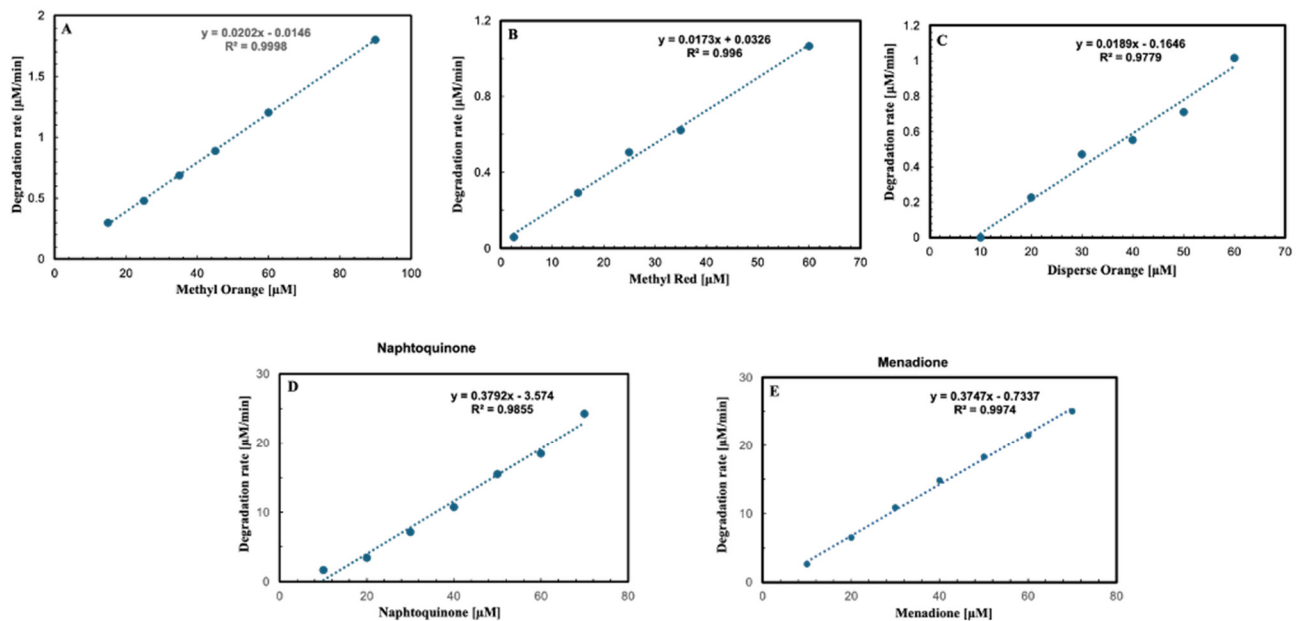


Figure S2. Kinetic analysis of YwqN for Azo dyes and quinone compounds. The kinetic analysis was performed with different concentration of substrates: A) Methyl Orange B) Methyl Red C) Disperse Orange D) Naphtoquinone E) Menadione. The degradation rate was calculated as the dye concentration (µM) processed by YwqN/min as indicated in Materials and Methods.

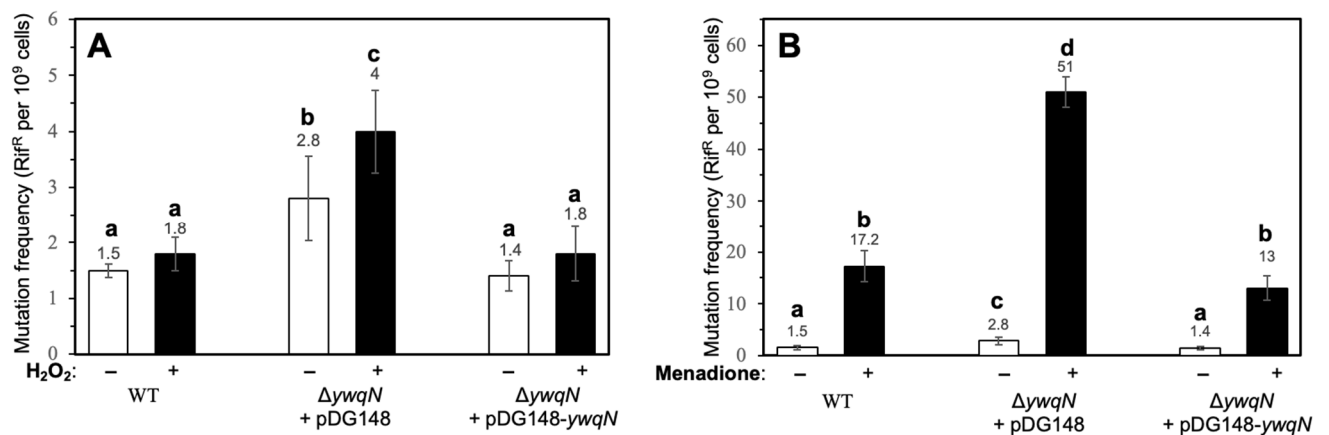


Figure S3. Complementation of spontaneous or H₂O₂-induced (A) and MD-induced (B) mutation frequencies following expression of *ywqN* from an IPTG-inducible expression vector. Cells of strain $\Delta ywqN$ carrying, where indicated, a pDG148-*Phs-ywqN* construct or the empty pDG148 vector were propagated in A3 medium in the absence or presence of H₂O₂ or menadione and frequencies of mutation to Rif^R were determined as described in Materials and Methods. Each bar represents the mean of data collected from three independent

experiments done in sextuplicate, and the error bars represent standard errors of the means (SEM). Mutation frequencies of all strains untreated and treated were compared with a Mann-Whitney-Wilcoxon U-Test, with a level of significance of 95% ($p < 0.05$). Different letters indicate statistically significant differences.

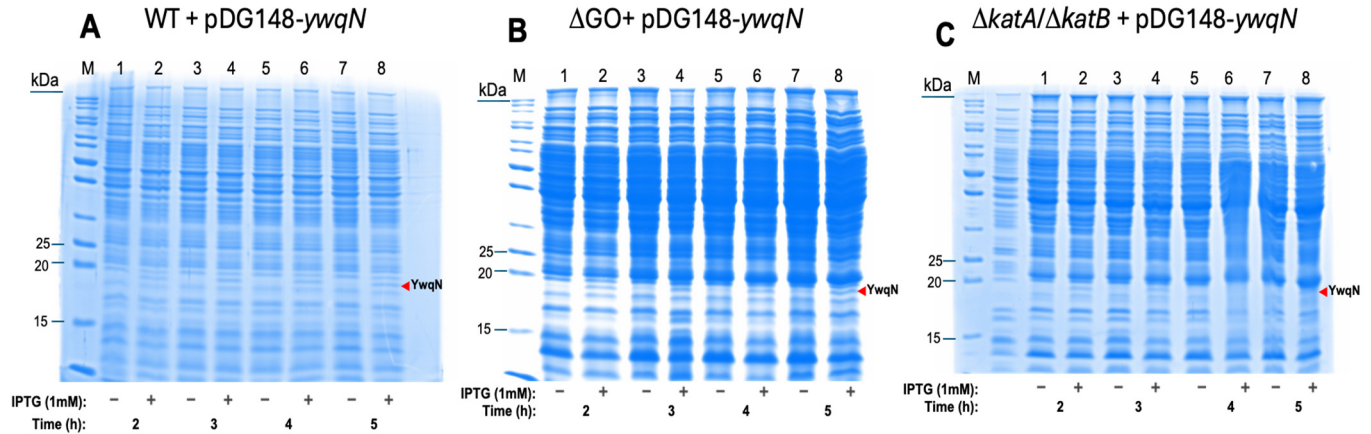


Figure S4. SDS-PAGE analysis of YwqN expression in *B. subtilis* WT (A) and strains deficient for the GO system (B) or KatA/KatB (C). All the strains were propagated in LB medium at 200 rpm/37°C, to an OD₆₀₀ of 0.5. At this point, the cultures were split in two Erlenmeyer flasks, one the flasks were amended with 1 mM IPTG to induce expression of *ywqN* and the second one was left untreated. All the cultures were incubated at 37°C and shaken at 200 rpm. Cell samples were collected during the times indicated and disrupted by sonication. Proteins from soluble-cell free extracts were visualized by Coomassie Blue staining. Aliquots of 75 μ L of each induction condition were loaded to the polyacrylamide gel.

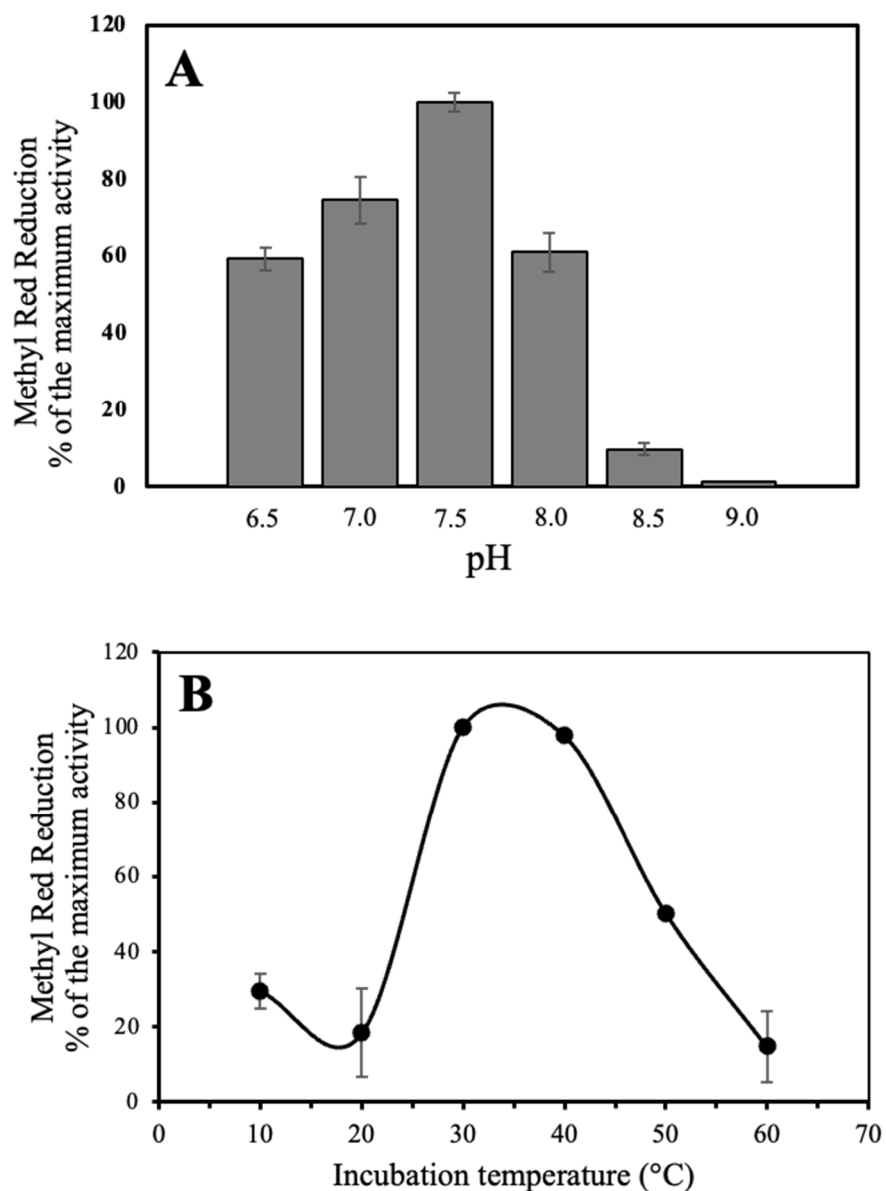


Figure S5. Optimal temperature (A) and pH (B) for His₆-YwqN-promoted methyl red reduction. Reaction mixtures containing 0.5 μ M of His₆-YwqN, 1 mM NADPH and 100 μ M methyl red were set to determinate the optimal pH (A) and temperature (B) for maximal enzyme activity of His₆-YwqN, plotted as percentage of maximal activity of methyl red reduction. The values plotted are averages of two independent experiments $\pm$ SDs.