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Supplemental information

Characterization and mechanism investigation of salt-activated methionine sulfoxide reductase A from halophiles

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Supplemental Figures and Tables

1. **Figure S1** SDS-PAGE gel analysis of the recombinant expression of *Hh*MsrA and its variants. Related to Figure 1 and 6.
2. **Figure S2** Specific activities of *pm*MsrA under various concentrations of KCl. Related to Figure 1.
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6. **Figure S6** Half-life profiles of variant *Hh*MsrA-R168K in the presence and absence of KCl. Related to Figure 6.

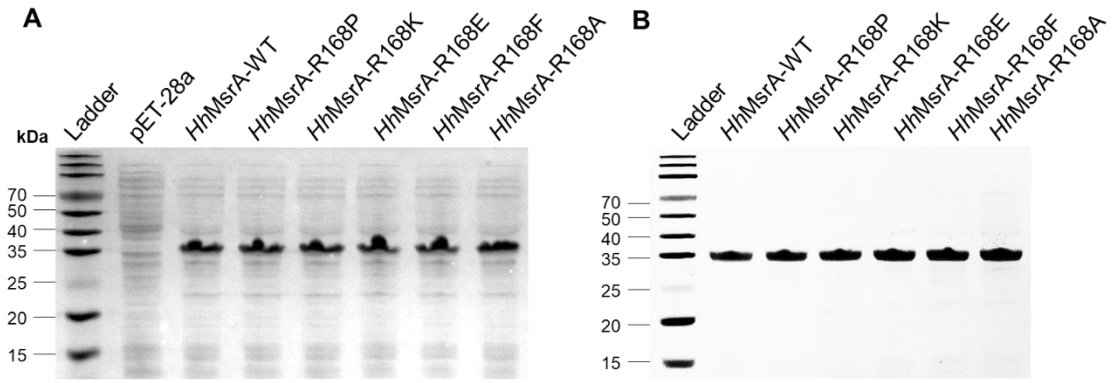


Figure S1 SDS-PAGE gel analysis of the recombinant expression of *Hh*MsrA and its variants. Thirty μg of soluble total proteins (A) and five μg of pure enzymes (B) were subjected to SDS-PAGE gel electrophoresis.

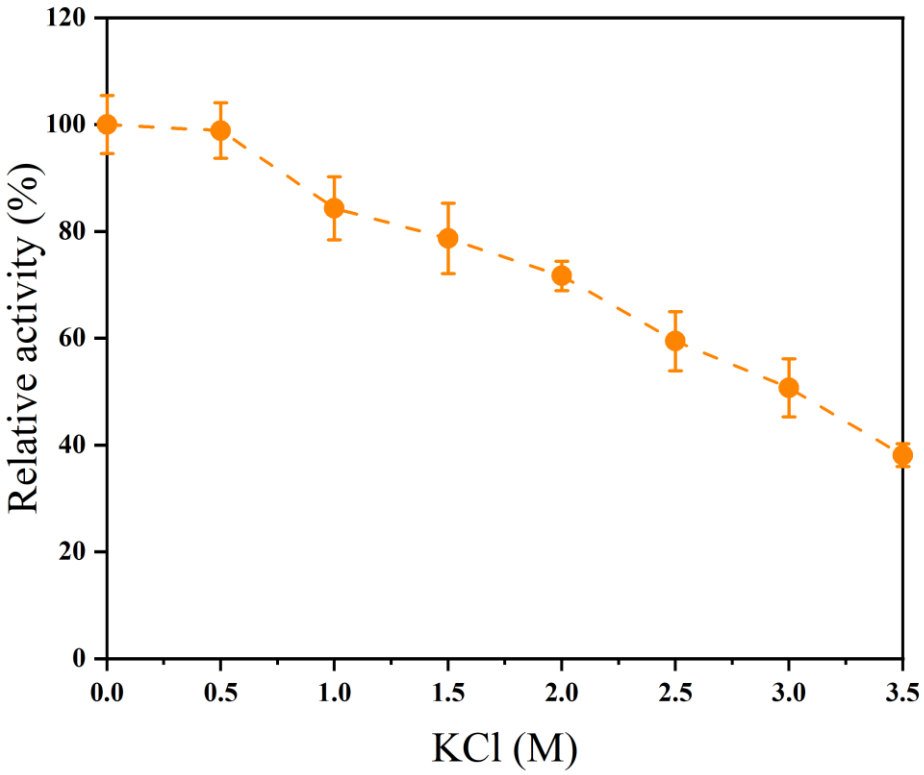


Figure S2 Specific activities of *pm*MsrA under various concentrations of KCl. The activities were assayed through the DTT-DTNB coupled colorimetric method. Each reaction was performed in the presence of 3 μM of pure enzyme, 3 mM of Met-O, and 1 mM of DTT for 45 min. Each value represents the mean ± SD of three independent experiments.

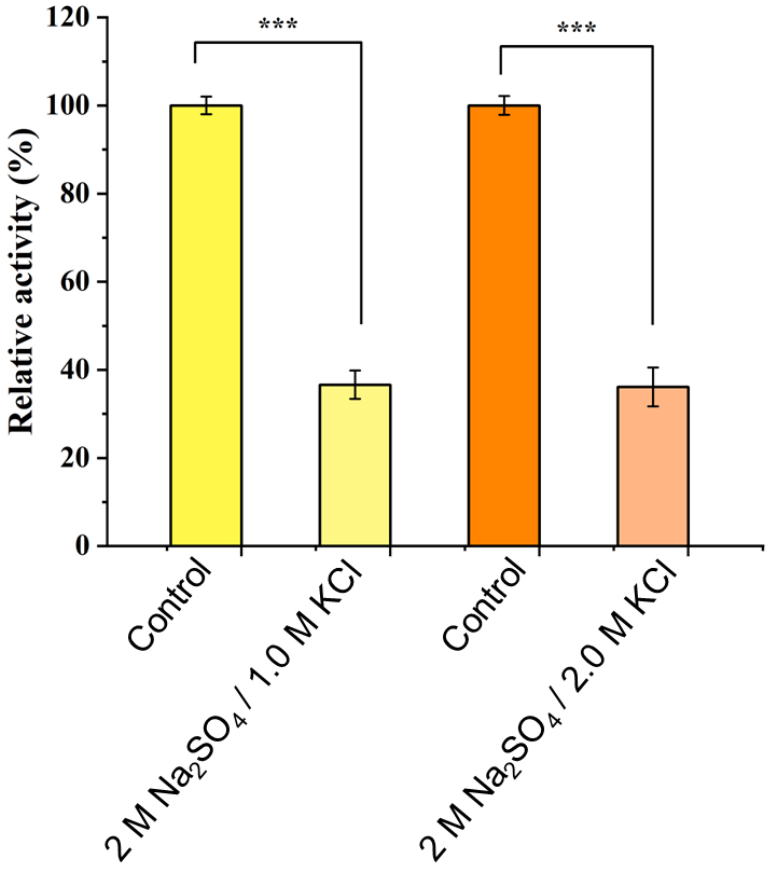
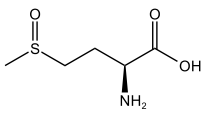
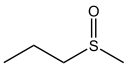


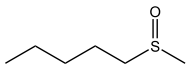
Figure S3 Investigation of the irreversible inhibition effects of sulfates on *Hh*MsrA activity. The *Hh*MsrA was first treated in a solution containing 2 M of Na_2SO_4 for 45 min. The enzyme was then washed, and its activity was measured under 1 M and 2 M KCl conditions (pH 10.0, 40 °C). Each value represents the mean ± SD of three independent experiments. *** represents $p < 0.001$.



Methionine sulfoxide



1-(methylsulfinyl)propane



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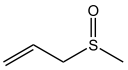
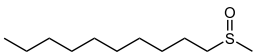


3-(methylsulfinyl)prop-1-ene



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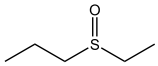

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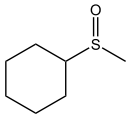

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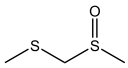
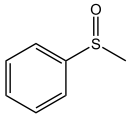
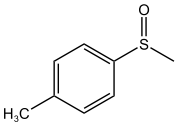


Methanesulfinyl-methylsulfanyl-methane



(methylsulfinyl)benzene



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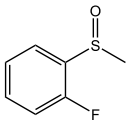

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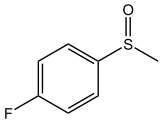

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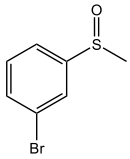

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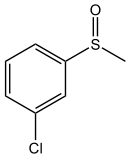

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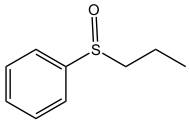
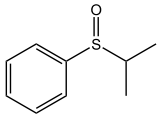
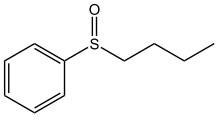


(propylsulfinyl)benzene



(isopropylsulfinyl)benzene



(butylsulfinyl)benzene

Figure S4 Substrates structure for specificity analysis of *Hh*MsrA

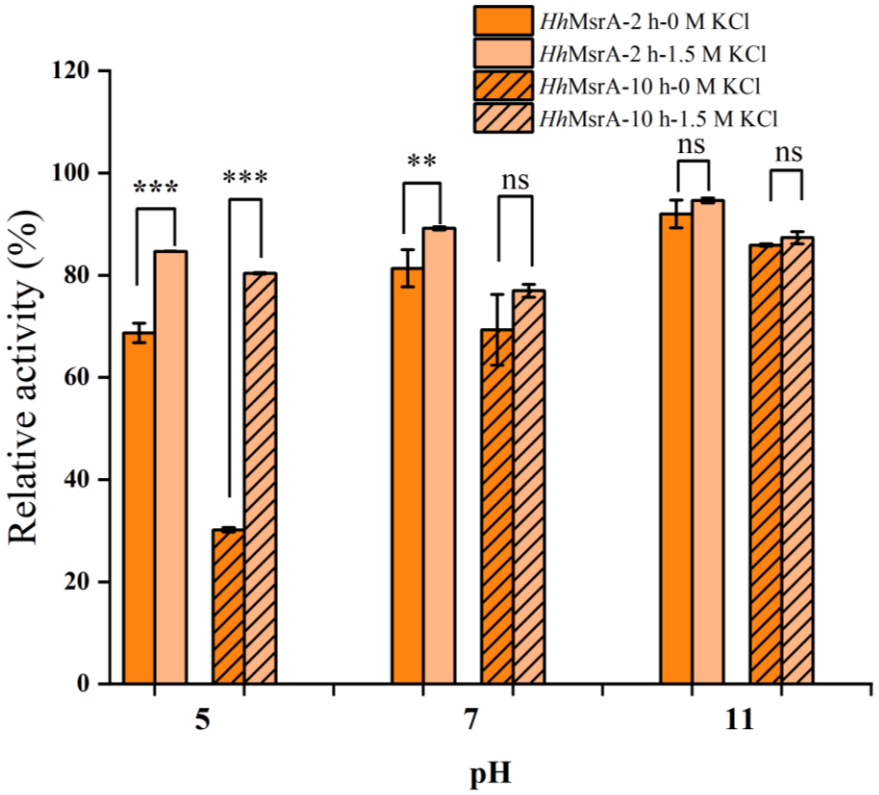


Figure S5 pH stability profile of *Hh*MsrA with and without KCl. The *Hh*MsrA was first treated in solutions with pH 5, 7, and 11 for 2 h or 10 h, in the absence and presence of 1.5 M KCl. The enzyme activity was then measured under conditions of 40 °C, pH 10.0, and 1.5 M KCl. Each value represents the mean ± SD of three independent experiments. ** represents $p < 0.01$, *** represents $p < 0.001$.

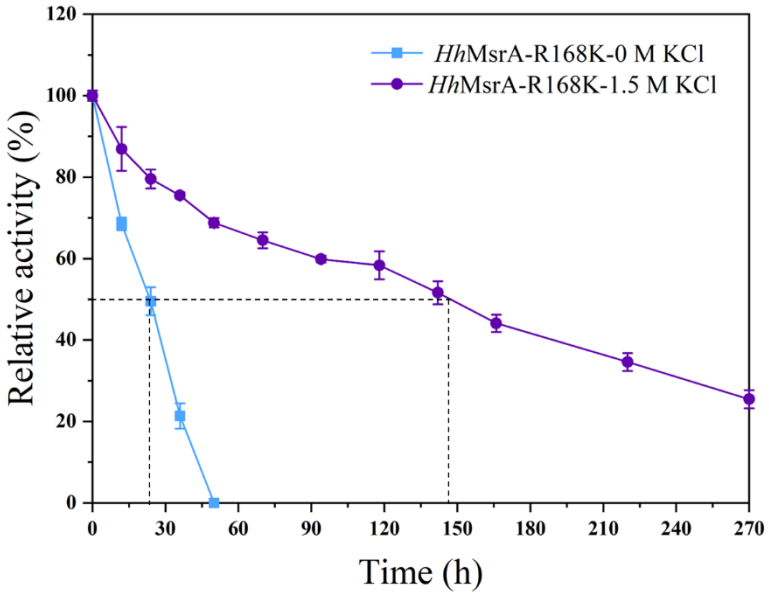


Figure S6 Half-life profiles of variant *Hh*MsrA-R168K in the presence and absence of KCl. The enzyme was first treated at 30 °C from 0 h to 270 h, in the absence and presence of 1.5 M KCl. The enzyme activity was then measured under conditions of 40 °C, pH 10.0, and 1.5 M KCl. Each value represents the mean ± SD of three independent experiments.

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