

Figure S1. Analysis of the interaction between the wild type SiRe\_0427 and RadA proteins by gel filtration. (A) Purified wild type SiRe\_0427 (5.2  $\mu$ M) and RadA (6.4  $\mu$ M) were used for the analysis. RadA and SiRe\_0427 eluted at 9.35 ml and 10.5 ml, respectively in separate peaks. (B). SDS-PAGE (15%) analysis of the fractions. M, molecular size markers.

B



Figure S2. SiRe\_0427 and CgLOG have no enzyme activity on cAMP, ATP, ADP, UMP, GMP, and CMP substrates. The enzyme activities of wild type SiRe\_0427 and CgLOG were checked on cAMP (A), ATP (B), ADP (C), UMP (D), GMP (E), and CMP (F). The 20  $\mu$ l reaction mixtures for various substrates were prepared using 6.4  $\mu$ M of SiRe\_0427 at pH 7.4 (or 8  $\mu$ M of CgLOG at pH 8.0) and 20 mM of each of the respective substrates. The mixtures were incubated at either 65°C or 37°C (CgLOG) for a specified period of time (see Materials and Methods). One to two microliters of the mixture samples were dotted on thin layer chromatography (TLC) sheet and the substrate and and products were visualized under UV lamp at 254 nm. The adenine, cAMP, ATP, ADP, UMP, GMP, CMP, and uracil controls are indicated on top and left of the figures. Reaction time (h) is indicated at the bottom of each figure.



B



Figure S3. SiRe 0427 exhibits phosphoribohydrolase activity on AMP. (A) Comparison of the activity of SiRe 0427 with Corynebacterium glutamicum LOG protein (CgLOG). The time course of the activity was measured by incubating 18 µM CgLOG in 20  $\mu$ l reaction mixtures (pH 8.0) at 37° C. (B) Quantification of the results in (A) using ImagQuant. Briefly, 2 µl aliquots of the samples were dotted on thin layer chromatography (TLC), dried, and visualized under a UV lamp at 254 nm. The AMP and adenine standards are in the left two lanes. The reaction time (hours) is indicated at the bottom of the figure. All experiments were repeated at least three times.



Figure S4. SiRe\_0427 exhibits phosphoribohyrolase activity on AMP at a wide range of temperatures. (A) The activity of the wild type SiRe\_0427 protein was measured at increasing temperature gradient. The wild type protein with concentration of 6.4  $\mu$ M was incubated at increasing temperature gradient (25, 35, 45, 55, 65, 75, and 85°C) at pH 7.4 for 3 hrs. One to two microliters of the mixture samples were dotted on TLC sheet and the substrate and and products were visualized under a UV lamp at 254 nm. (B) The quantified result of (A). The adenosine 5'mononphosphate (AMP) and adenine are indicated on the left side of the figure.



Figure S5. Three-dimensional model of SiRe 0427 protein (monomer) generated by **SWISS\_MODEL Server.** The secondary alpha ( $\alpha$ ) helices (cyan) and beta ( $\beta$ ) sheets (pink) are numbered. The structure is composed of six alpha helices and seven beta sheets typical of Rossmann fold  $\alpha/\beta$  arrangement. G101, G102, G103, G105, T106, and E109 (green) in the "GGGxGTxxE" motif located between ( $\beta$ 5-  $\alpha$ 4) are shown with stick models. Other conserved residues G37, G38, and M43 (green) are also shown. The proximity and the spatial arrangement of the atoms involved in enzyme catalysis are indicated as rectangular black box. The residues are labeled and indicated as ball and stick models. (B) Structural comparison of SiRe 0427 with Corynebacterium glutamicum LOG II (CgLOG II) (PDB: 5WQ3) using the pyMOL server. The "P/GGGxGTxxE" motifs between the two structures are indicated on the figure. The catalytic residues are shown in orange. Green, SiRe 0427; Cyan, CgLOG.



Figure S6. The tetrameric model of SiRe\_0427 protein developed by SWISS-MODEL server and visualized in pyMOL server (<u>http://www.pymol.org</u>). The four modeled chains are indicated with numerals i, ii, iii, and iv. The enzyme catalytic residues Arg86 and Glu109 are indicated by an arrow positioned between each of the two residues.



**Figure S7. Analysis of pMID**-*sire\_0427*-**T transformant strain by PCR using 0427**-**Flanking primers**. (A) Schematic diagram representing the lengths of the target gene and marker gene integrated into the host genome compared to the wild type genome. (B) Gene fragments amplified by PCR using 0427-Flanking forward/reverse primers were analyzed in a 1 % agarose gel.



Figure S8. Structural comparison of the monomers of SisLOG (SiRe\_0427), CgLOG I (PDB: 5ITS), and CgLOG II (PDB: 5WQ3) using the pyMOL server. SisLOG (Green), CgLOG I (Cyan), and CgLOG II (Magenta) are superposed. CgLOG II contains an additional helix. The conserved nucleotide binding motif "P/GGGxGT" and catalytic residues of the LOGs are indicated.



**Figure S9. Identification of residues involved in subunit interaction of CgLOG.** The two subunits are shown in green and cyan, respectively. The yellow dotted lines indicate the interacting residues.



Figure S10. Comparison of residues involved in the oligomer formation of SisLOG (green) and CgLOG I (cyan, PDB: 5ITS).



Figure S11. A model for the oligomer formation of different LOGs.