Structural and Enzymatic characterization of the lactonase SisLac from Sulfolobus islandicus

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Abstract

**Background:** A new member of the Phosphotriesterase-Like Lactonases (PLL) family from the hyperthermophilic archeon *Sulfolobus islandicus* (SisLac) has been characterized. SisLac is a native lactonase that exhibits a high promiscuous phosphotriesterase activity. SisLac thus represents a promising target for engineering studies, exhibiting both detoxification and bacterial *quorum* quenching abilities, including human pathogens such as *Pseudomonas aeruginosa*.

**Methodology/Principal Findings:** Here, we describe the substrate specificity of SisLac, providing extensive kinetic studies performed with various phosphotriesters, esters, N-acyl-homoserine lactones (AHLs) and other lactones as substrates. Moreover, we solved the X-ray structure of SisLac and structural comparisons with the closely related *Sso*Pox structure highlighted differences in the surface salt bridge network and the dimerization interface. SisLac and *Sso*Pox being close homologues (91% sequence identity), we undertook a mutational study to decipher these structural differences and their putative consequences on the stability and the catalytic properties of these proteins.

**Conclusions/Significance:** We show that SisLac is a very proficient lactonase against aroma lactones and AHLs as substrates. Hence, data herein emphasize the potential role of SisLac as *quorum* quenching agent in *Sulfolobus*. Moreover, despite the very high sequence homology with *Sso*Pox, we highlight key epistatic substitutions that influence the enzyme stability and activity.

Introduction

SisLac (also known as *Sso*Pox [1]) is an enzyme isolated from the archaean organism *Sulfolobus islandicus*, which is found in extreme environments like the Yellowstone natural park (U.S.A.) or the Mutnovsky volcano in Kamchatka (Russia) [2]. SisLac belongs to an enzyme family called Phosphotriesterase-Like Lactonase (PLL) that encompasses members from mesophilic organisms (PH, AhLA, MCP, *DOPH*) [3,4,5] as well as thermophilic (*Sso*Pox, *Sso*Lac, *Gp*IP, *Gk*L) [6,7,8,9] representatives. The PLL family is structurally and biochemically related to bacterial Phosphotriesterases (PTEs) [3]. Indeed, some representatives of PLLs were primarily isolated by virtue of their phosphotriesterase activity towards the insecticide paraoxon, and were named paraoxonases (PTEs) [6]. However, further phylogenetic and biochemical studies has revealed that these enzymes are native lactonases endowed with promiscuous paraoxonase activity and more generally with organophosphate hydrolase activity [3]. Hyperthermophilic PLLs (hPLLs) are appealing enzymes in biotechnology because they possess an intrinsically high stability that often confer high resistance towards harsh conditions and proteases activity [10], which constitute useful properties for storage and large scale purification.

Interestingly, PTEs exhibit diffusion limit-like second order rates with paraoxon as a substrate [11], and are also endowed with promiscuous lactonase activity [3,12]. The particular link between these two families raises the hypothesis that PTEs have diverged from native lactonases like PLLs [3,13,14,15]. Indeed, both PTEs and PLLs belong to the amidohydrolase superfamily [3]. Despite the relatively low sequence identity between these two families (~30%), PTEs and PLLs exhibit the same β/α barrel fold or so-called TIM barrel [16]. At the C-terminus of the barrel, two divalent metal cations constitute the active site [13,17,18]. The phosphotriesterase activity of PTEs and PLLs is modulated in the presence of various divalent cations, the highest activity being achieved in a cobalt-containing buffer for *Pseudomonas diminuta* PTE [19], *OpdA* [20] and *Sso*Pox [6]. The active site metal cations’ chemical nature has been investigated using anomalous X-ray scattering and has revealed that both the PTE from *Agrobacterium radiobacter* (*OpdA*) and the PLL *Sso*Pox possess an iron/cobalt heteronuclear center when the expression media is supplemented with cobalt ions [13,20].
The catalytic mechanisms for both the lactonase and phosphotriesterase involve a nucleophilic attack by a water molecule activated by the bi-metallic center. The major difference between the two activities consists of the respective transition state geometries: bi-pyramidal for the phosphotriesterases and tetrahedral for the lactones. The fact that these two activities can be catalyzed with significant rates within the same active site suggests an overlap between the stabilization of the corresponding transitions state species, from which the enzymatic promiscuity would stem from [14,21]. The active site of PLLs possesses three sub-sites that are remarkably adapted for the lactone binding: a small sub-site, a large sub-site and a hydrophobic channel [13]. The aliphatic chain of the lactones binds within the hydrophobic channel, the large sub-site accommodates the amide group of the N-acyl chain, and the small sub-site positions the lactone ring. AHLs are molecules that mediate bacterial communication (quorum sensing) for many species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species. These molecules accumulate in the media to reach a certain threshold for which the transcriptional profile of the species.

Production-purification of SisLac, SsoPox and SisLac’s Variants

SisLac and its variants were heterologously produced and purified from the Escherichia coli strain BL21(DE3)-pGro7/GroEL (TaKaRa) as previously described [1] with the only difference being that 0.2% (w/v) arabinose was added at the start of the over-expression in order to induce chaperones expression. A very similar production protocol was used for wt SisPox. Briefly, protein production was performed in 2 liters of ZYP medium [35] (100 μg/ml ampicillin, 34 μg/ml chloramphenicol) inoculated by over-night pre-culture at a 1/20 ratio. Cultures grew at 37°C to reach OD600nm = 1.5. The induction of protein production was made by starting the consumption of the lactose in ZYP medium. Subsequently, 0.2 mM CoCl2 was added and the temperature was reduced to 25°C for additional 20 hours. Cells were harvested by centrifugation (3 000 g, 4°C, 10 min), re-suspended in lysis buffer (50 mM HEPES pH 8, 150 mM NaCl, CoCl2 0.2 mM, Lysozyme 0.25 mg/ml, PMSF 0.1 mM DNaseI 10 μg/ml) and stored at −80°C. The frozen cells were thawed and disrupted by three steps of centrifugation (12 000 g, 4°C, 30 min). The purified protein being hyperthermostable, host proteins were precipitated by incubation of 30 min at 70°C and harvested by centrifugation (12 000 g, 4°C, 30 min). Other thermostable proteins from the host E. coli were eliminated by ammonium sulfate precipitation (326 g/L), and the overexpressed protein was concentrated by ammonium sulfate precipitation (476 g/L) and suspended in activity buffer (HEPES 50 mM pH 8, NaCl 150 mM, CoCl2 0.2 mM). The remaining ammonium sulfate was removed by dialysis against the activity buffer and the protein sample was subsequently concentrated prior to the size exclusion chromatography step (S75-16-60, GE Healthcare). The yield of protein production varied between 20 and 100 mg of protein per liter of culture after purification. The purity and the protein quality were verified by SDS-PAGE and mass spectrometry.

Materials and Methods

Strain, Plasmids and Site Directed Mutagenesis

The plasmids preparations were performed in Escherichia coli strain DH5α (Invitrogen). Protein production was performed in E. coli BL21(DE3)-pGro7/GroEL strain (TaKaRa) using plasmids pET22b-StrepTevSisLac and pET22b-SsoPox (provided by GenecoArt; Germany). Site directed mutagenesis was performed in 50 μL using Pfu polymerase (Invitrogen) on 100 ng of plasmid encoding corresponding genes and primers referenced in Table S1. The PCR cycle was performed using hybridization temperature of 55°C, elongation time of 12 min during 30 cycles and final elongation of 20 min. The template plasmid was eliminated by Fast Digest DpnI (Fermentas) digestion of 30 min at 37°C followed by inactivation step of 20 min at 80°C. Plasmids were concentrated by classical alcohol precipitation and then electro-porated (Gene-Pulser, Bio-Rad) into E. coli strain S1pho cells (Lucigen), a particularly competent strain of E. coli. Site directed mutagenesis was finally verified by sequencing.

Multi-angle Light Scattering Studies: experiments were performed at room temperature using zetasizer nano series apparatus (Malvern, UK) and the Zetasizer software. 30 μL of purified wt SsoPox and SisLac (2.5 mg/mL) was used in the activity buffer to measure the hydrodynamic radius of particles in the protein solutions at 633 nm.
Enzymatic Characterization

The time course of ethyl-paraoxon hydrolysis by SisLac at 70°C was monitored following the p-nitrophenololate production at 405 nm ($\varepsilon_{405nm} = 17,000 \text{ M}^{-1} \text{cm}^{-1}$) in 1-cm path length cell with a Cary WinUV spectrophotometer (Varian, Australia) and using the Cary WinUV software. Standard assays (500 μL) were performed in paraoxonase buffer CHES 50 mM pH 9, NaCl 150 mM, CoCl$_2$ 0.2 mM, EtOH 6% (v/v), with pH adjusted with NaOH at 70°C.

At 25°C, the phosphotriesterase, esterase and lactonase activities were analyzed by monitoring absorbance variations in 200 μL reaction volumes using 96-well plates (6.2-mm path length cell) and a microplate reader (Synergy HT) using the Gen5.1 software at 25°C. For each substrate, assays were performed using organic solvent concentrations below 1%. The monitoring wavelength, the solvent used, the molar extinction coefficient and the concentration range for each substrate (Fig. 1, S1 & S2) are summarized in Table S2. Phosphotriesterase and esterase activities were performed in activity buffer. When required, DTNB at 2 mM was added to the buffer to follow hydrolysis of substrate releasing thiolate group (malathion (Fig. S1IV)). Catalytic parameters for some phosphotriesterases were also recorded using SDS at concentrations 0.01 and 0.1% (w/v). Lactone hydrolysis assays were performed in lactonase buffer (Bicine 2.5 mM pH 8.3, NaCl 150 mM, CoCl$_2$ 0.2 mM, Cresol purple 0.25 mM and 0.5% DMSO) using cresol purple (pKa 8.3 at 25°C) as pH indicator to follow the acidification related to the lactone ring hydrolysis. Molar coefficient extinction was measured by recording absorbance of the buffer over a range of acetic acid concentrations (0–0.35 mM). The absorbance values versus acetic acid concentration were fitted to a linear regression (GraphPad Prism 5 software) with a slope corresponding to molar extinction coefficient (see Table S2).

Thermostability Analysis

**Temperature dependence analysis.** The temperature dependence of the SisLac paraoxonase activity was studied over the temperature range 25–85°C with 10°C increment. The ethyl-paraoxon (2 mM) hydrolysis was monitored in 500 μL at 405 nm ($\varepsilon_{405nm} = 17,000 \text{ M}^{-1} \text{cm}^{-1}$) in 1-cm path length cell with a Cary WinUV spectrophotometer (Varian, Australia) using the Cary WinUV software. Triplicate experiments were performed in paraoxonase buffer with pH adjusted with NaOH to 9 at each temperature.

Activity-based thermal stability. The residual paraoxonase activity of SisLac after incubation at different temperatures was performed. Incubation time and temperatures tested in this experiment were 390 min at 85°C, 90°C and 95°C in activity buffer. The ethyl-paraoxon (1 mM) activity was followed at 25°C every 15 min during the first hour of incubation and every 30 min till the end of the experiment (after cooling). Paraoxon hydrolysis was monitored at 405 nm ($\varepsilon_{405nm} = 17,000 \text{ M}^{-1} \text{cm}^{-1}$) for 30 min with a Cary WinUV spectrophotometer (Varian, Australia) using the Cary WinUV software. Gen5.1 software was used to evaluate the initial velocity at each temperature.

**Circular Dichroism** (CD) spectra were recorded using Jasco J-810 spectropolarimeter equipped with a Jasco PTC-4235 in 1 mm thick quartz cell and using the Spectra Manager software. Measurements were carried out in 10 mM sodium phosphate buffer at pH 8 with a protein concentration of 0.1 mg/mL. For wt SisLac, denaturation was first recorded between 190 to 260 nm with a scattering speed of 20 nm/min every 10°C at temperatures ranging between 20 to 90°C. To determine the melting temperature of proteins (wt SisLac and variants), the denaturation was recorded at 222 nm by increasing the temperature from 20 to 90°C (at 5°C/min) in 10 mM sodium phosphate Buffer at pH 8 containing increasing concentrations (0.5–3.5 M) of guanidinium chloride. The theoretical Tm without guanidinium chloride was extrapolated at the y-intercept by a linear fit using the GraphPadPrism 5 software.

**pH Dependence Profile Determination**

The pH-dependence ethyl-paraoxonase activity (2 mM) profile of SisLac was monitored at 348 nm (the pH-independent isobestic point of p-nitrophenol and p-nitrophenoxide ion; $\varepsilon_{440nm} = 5300 \text{ M}^{-1} \text{cm}^{-1}$) [6]. To explore the pH range 5–11, different buffers were prepared containing 50 mM monobasic phosphate over the pH range 5–7, 50 mM HEPES for pH 8, 50 mM CHES as pH indicator to follow the activity buffer (50 mM HEPES for pH 7, 50 mM CHES for pH 8, 50 mM Mes for pH 6)
Crystallization and Structure Determination

The crystallization procedure of SisLac has been previously described [1]. Diffraction data were collected at the ESRF (Grenoble, France) BM-30A (FIP) beamline using a wavelength of 0.98 Å on an ADSC Quantum Q315 Detector. X-Ray diffraction data were integrated and scaled with the XDS program [36]. The presence of a twin was clearly established using phenix.xtriage [39]. The molecular replacement using the SsoPox structure as model (PDB code 2vc5) was performed with Phaser [39]. The twin operator (h,k,l) and a twofold axis (z) arising from the twinning were determined using phenix.xtriage. The solution was then used for refinement performed using REFMAC5 [40] using the twin option and Cost [41] for model improvement. The model and structure factor were deposited under the Protein Data Bank (PDB) code 4G2D. Despite the twinning, the electron density maps were of good quality (Fig. S3; R and Rfree values 0.2649 and 0.2925, respectively; Table 1).

Structural analysis and comparison, cartoon and ribbon representation were made using PyMOL (www.pymol.org). Surface contacts and interaction analysis was performed using the PROTORF server [42]. Root mean square deviations (r.m.s.d) were computed using Swiss pdb-viewer software [43].

Sequence Alignment

The alignment was performed using T-coffee server [44,45], manually improved with seaview software [46] and finally drawn with BioEdit 7.1.3.

Results

This study provides the characterization of SisLac isolated from Sulfolobus islandicus strain M.16.4. Several genomes of S. islandicus are available and encode highly similar (99% sequence identity) orthologs of SisLac (Fig. S4). Sequence comparison with close homologs SsoPox [91% identical] from Sulfolobus solfataricus MT4 & P2 [6] and SsoPox (76% identical) from Sulfolobus acidocaldarius DSM 639 [7] (Fig. 2 & S4) reveals that the sequence divergence is mainly localized at the N-terminus and C-terminus of the protein. SisLac displays lower sequence identity with other members of the PLL family of proteins (35% Gp/Gl from Geobacillus sp. [8,9], 28% DOOPH form Deinococcus radiodurans [5,34], 37–38% AhLA/PPH/MCP from mesophilic organisms [3,4] and ~30% with PTEs [Fig. 2]. Despite a very high sequence homology between SsoPox and SisLac, both enzymes exhibit structural and enzymatic differences.

Biochemical and Biophysical Characterization of SisLac

Oligomeric state analysis. Size exclusion chromatography, dynamic light scattering and multi angle light scattering experiments were carried out to determine the oligomeric states of SisLac and SsoPox. Using a combination of static and dynamic light scattering, UV spectrophotometry, and refractometry, SisLac and SsoPox appear to be dimeric at room temperature (25°C) (Fig. 3A-B, 72.57±0.79 kDa and 70.46±0.97 kDa, respectively; versus MWs of SsoPox and SisLac dimers 71.2 kDa). Moreover, the dynamic light scattering experiments show apparent sizes for SisLac and SsoPox of 80±3 kDa and 82±3 kDa, respectively (Fig. 3C-D). These results confirm that both proteins are dimeric at 25°C. The existence of homodimers is consistent with the crystal structures of SisLac, SsoPox [47] and other PLLs (DOOPH (PDB ID: 3FDK), Gp (PDB ID: 3F4D) and Glk (PDB ID: 3OJG)).

pH and temperature dependence. We determined the pH and temperature dependence of SisLac’s catalytic activities. However, because the lactonase assay utilizes a pH indicator, these characteristics could only be determined for the paraoxonase activity. The optimal pH for SisLac’s paraoxonase activity was established by measuring the velocity at pH ranging from 5 to 11 (Fig. 4A). The pH-rate dependence plot displays a bell-shape curve with a wide plateau between pH 7 and 10 (with maximal activity at pH 9). The same dependency what was described for SsoPox at 70°C [7], and only a slightly different pattern was observed for SisLac (optimum at pH 8 [6]). The PTE from P.

Table 1. Data collection and refinement statistics of SisLac structure.

| Data collection |   |
|-----------------|---|
| PDB id          | 4G2D |
| Wavelength (Å)  | 0.980 |
| Detector        | ADSC Q315 |
| Oscillation (%) | 0.5 |
| Number of frames| 323  |
| Resolution (Å) (last bin) | 2.70 (2.80-2.70) |
| Space group     | P3_2_1 |
| Unit-cell parameters (Å) | a = 47.8, b = 47.8, c = 239.5 |
|                  | α = 90.0, β = 90.0, γ = 120.0 |
| No. of observed reflections (last bin) | 86521 (8903) |
| No. of unique reflections (last bin) | 9436 (959) |
| Completeness (%) (last bin) | 99.9 (100) |
| Rmerge (%) (last bin) | 6.2 (47.6) |
| Rfree (%) (last bin) | 6.6 (50.4) |
| I/σ(I) (last bin) | 28.42 (4.71) |
| Redundancy (last bin) | 9.17 (9.28) |
| Mosaicity (%) | 0.103 |

Refinement statistics

| Rfree/Rwork (%) | 29.25/26.49 |
|-----------------|-------------|
| No. of total model atoms | 2517 |
| Ramachandran favored | 98.2% |
| Ramachandran outliers | 1.8% |
| RMS from ideal | 0.0021 |
| Bond angles (%) | 0.5114 |
| a Rmerge = \sum_{i,j} | \frac{|F_{o}(i)-F_{c}(i)|}{\sqrt{\hat{F}_{o}(i)^2 + \hat{F}_{c}(i)^2}} |
| b Rfree = \sum_{i,j} \left[ \frac{\sum_{k} |F_{o}(i)-F_{c}(i)|}{\sqrt{\sum_{k} \hat{F}_{o}(i)^2 + \hat{F}_{c}(i)^2}} \right] |
| c Rfree = \sum_{i} \left[ \frac{\sum_{k} |\sum_{j} F_{o}(i)-F_{c}(i)|}{\sqrt{\sum_{k} \hat{F}_{o}(i)^2 + \hat{F}_{c}(i)^2}} \right] |

Pox at 25°C were performed at pH 9). The same dependency what was described for SsoPox (optimum at pH 8 [6]). The solution was then used for refinement performed using REFMAC5 [40] using the twin option and Cost [41] for model improvement. The model and structure factor were deposited under the Protein Data Bank (PDB) code 4G2D. Despite the twinning, the electron density maps were of good quality (Fig. S3; R and Rfree values 0.2649 and 0.2925, respectively; Table 1).

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pH and temperature dependence. We determined the pH and temperature dependence of SisLac’s catalytic activities. However, because the lactonase assay utilizes a pH indicator, these characteristics could only be determined for the paraoxonase activity. The optimal pH for SisLac’s paraoxonase activity was established by measuring the velocity at pH ranging from 5 to 11 (Fig. 4A). The pH-rate dependence plot displays a bell-shape curve with a wide plateau between pH 7 and 10 (with maximal activity at pH 9). The same dependency what was described for SsoPox at 70°C [7], and only a slightly different pattern was observed for SisLac (optimum at pH 8 [6]). The PTE from P.
P. diminuta also exhibits an activity maximum at a pH range of 8–10 [48]. This observation is consistent with the hypothesis of common mechanism shared by these enzyme families.

The temperature dependency was investigated by measuring the paraoxonase activity at temperatures ranging from 25 to 85°C (Fig. 4B). The highest temperature tested (85°C) presented the highest velocity. However, within the tested temperature (imposed by technical limitations), we did not find a maximum. Similarly, no maxima were found in the cases of SsoPox [6], DrOPH [5] and GsP [8]. In contrast, SacPox presents a maximum activity around 70°C and the activity decrease above this temperature [7].

**Thermostability**

The thermostability of SisLac was evaluated based on its catalytic activity. The residual paraoxonase activity of the enzyme after different incubation times at 80, 90 and 95°C was measured (Fig. 4C). The enzyme exhibited respective half-lives of 84±20 min, 8.5±1.5 min and 3.6±0.4 min at 85, 90 and 95°C. In comparison, SsoPox exhibits a half-life of 4 hours at 95°C, and 90 min at 100°C [6], and SacPox exhibits a half-life of 5 min at 90°C [7] (from S. acidocaldarius, living temperatures from 55 to 85°C [49]).

The thermal stability of SisLac was also determined by circular dichroism at temperatures ranging between 20°C and 90°C (Fig. S5). However, as previously observed for SsoPox [47], the extreme thermostability of this enzyme does not allow to precisely determine a melting temperature (Tm) by this method. Different concentrations of guanidium chloride were required to further destabilize the protein and the Tm values were extrapolated to zero guanidinium chloride: Tm at 102±2°C for SisLac (Fig. S5) (while SsoPox’s Tm = 106°C [47]). These values are in the range of the other characterized thermostable PLLs, including DrOPH and GsP whose Tm values are 88.1°C [5] and 106.6°C [8], respectively. Notably, the extremophile Deinococcus radiodurans is a...
(5.47±0.47)×10^3 M^{-1}s^{-1}) [8], GbL (4.5 M^{-1}s^{-1}; at 37°C) [9], MCIP (4.1 M^{-1}s^{-1}) [4] and DOPH (1.39±0.11 M^{-1}s^{-1}) [5]. These promiscuous phosphotriesterase catalytic parameters of lactonases [3,14] contrast with the diffusion limit-like second order rates of P. diminuta PTE with paraoxon as substrate (k_{cat}/K_{M}~10^6 M^{-1}s^{-1}) [11].

The modulation of SiLac phosphotriester hydrolysis by Sodium Dodecyl Sulfate (SDS), which has been previously shown to act as an activator in the case of SoPox [53], was also tested (Table 2). Interestingly, the addition of 0.01% SDS yields to a 2.5 folds increase in the paraoxonase catalytic efficiency (6.36±2.18)×10^2 M^{-1}s^{-1}), whereas the addition of 0.1% SDS enhanced the efficiency by 25 folds (k_{cat}/K_{M}=7.14±4.16)×10^3 M^{-1}s^{-1}).

Others phosphotriesters (Fig. 1A) were also tested as substrates at 25°C; including methyl-paraoxon (Fig. S1II), parathion (Fig. S1III), methyl-paranitrophenol (Fig. S1IV), malathion (Fig. S1V) and CMP-coumarin (Fig. S1VI) [54]. The catalytic efficiency at 25°C for (ethyl-)paraoxon was 6.36±2.18)×10^2 M^{-1}s^{-1}) and is higher than that of GbL [8] (4.5 M^{-1}s^{-1}; at 37°C) [9]. MCIP (4.1 M^{-1}s^{-1}) [4] and DOPH (1.39±0.11 M^{-1}s^{-1}) [5].

Enzymatic Characterization of SiLac

Phosphotriesterase activity. Kinetic parameters were determined for paraoxon (Fig. S1I) at 70°C and 25°C (Table 2). The catalytic efficiency obtained at 70°C for SiLac (k_{cat}/K_{M}=6.98±1.16)×10^2 M^{-1}s^{-1}) is similar to that reported for SoPox [7] and SoPox [52] (Table S3). The catalytic efficiency at 25°C was about 2.5 folds lower than the efficiency at 70°C (2.60±0.58)×10^2 M^{-1}s^{-1}), and is higher than that of GbL (4.57±0.47)×10^3 M^{-1}s^{-1}) [8], GbL (4.5 M^{-1}s^{-1}; at 37°C) [9], MCIP (4.1 M^{-1}s^{-1}) [4] and DOPH (1.39±0.11 M^{-1}s^{-1}) [5]. These promiscuous phosphotriesterase catalytic parameters of lactonases [3,14] contrast with the diffusion limit-like second order rates of P. diminuta PTE with paraoxon as substrate (k_{cat}/K_{M}~10^6 M^{-1}s^{-1}) [11].

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Table 2. Ethyl-paraoxonase activity of SiLac.

| Conditions | SiLac |
|------------|-------|
|            | k_{cat} (s^{-1}) | k_{M} (µM) | k_{cat}/K_{M} (s^{-1} M^{-1}) |
| 25°C       | 1.42±0.09 | 5439±873 | 2.60±0.58×10^2 |
| 25°C SIS 0.1% | 14.31±3.16 | 2005±728 | 7.14±4.16×10^4 |
| 25°C SIS 0.01% | 2.70±0.29 | 4248±999 | 6.36±2.18×10^5 |
| 70°C       | 0.79±0.04 | 1131±196 | 6.98±1.56×10^5 |

Data obtained with cobalt as cofactor.

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Table 3. Phosphotriesterase activity of SisLac.

| Substrate                  | ScLac | SsoLac |
|----------------------------|-------|--------|
| Ethyl-paraoxon (I)         | 4.32 ± 0.09 | 5 ± 0.4 
| Ethyl-parathion (II)       | 7.40 ± 1.26 | 1.37 ± 0.41 |
| Methyl-paraoxon (III)      | ND    | ND     |
| Methyl-parathion (IV)      | 9.75 ± 0.2 | 2.17 ± 0.3 |
| Malathion (V)              | 6.2 ± 0.4 | 30.5 ± 5.4 |
| CMP (VI)                   | 1.86 ± 0.2 | 2 ± 0.3 |

Table 4. Esterase activity of SisLac.

| Substrate                  | ScLac | SsoLac |
|----------------------------|-------|--------|
| Ethyl-paraoxon (I)         | ND    | ND     |
| Ethyl-parathion (II)       | ND    | ND     |
| Methyl-paraoxon (III)      | ND    | ND     |
| Methyl-parathion (IV)      | 2.78 ± 0.3 | 2.17 ± 0.3 |
| Malathion (V)              | 6.2 ± 0.4 | 30.5 ± 5.4 |
| CMP (VI)                   | 1.86 ± 0.2 | 2 ± 0.3 |

Table 5. Lactonase activity of SisLac.

| Substrate                  | ScLac | SsoLac |
|----------------------------|-------|--------|
| Ethyl-paraoxon (I)         | 4.32 ± 0.09 | 5 ± 0.4 |
| Ethyl-parathion (II)       | 7.40 ± 1.26 | 1.37 ± 0.41 |
| Methyl-paraoxon (III)      | ND    | ND     |
| Methyl-parathion (IV)      | 9.75 ± 0.2 | 2.17 ± 0.3 |
| Malathion (V)              | 6.2 ± 0.4 | 30.5 ± 5.4 |
| CMP (VI)                   | 1.86 ± 0.2 | 2 ± 0.3 |

Table 6. Others lactones were also assayed as substrates (Table 5), such as the γ-lactones (5 atoms lactone ring) (Fig. 1E), δ-lactones (6-atoms lactone ring) (Fig. 1F) and ε-lactone (7 atoms lactone ring) (Fig. 2X), with alkyl substituent on carbons 4 and 5 of the lactone ring (contrary to the substitution of carbon 2 in AHLs (Fig. 1C). Finally, dihydrocoumarin (Fig. 2XI), an aromatic lactone, was also tested (Table 6). We found that γ-lactones (Fig. 1E) and δ-lactones (Fig. 1F) comprise good substrates for SisLac, δ-lactones being the preferred substrates. Indeed, the best δ-lactone (undecanoic-δ-lactone (Fig. 2XVI), 1.77 ± 0.04 × 10^6 M^-1 s^-1) is hydrolyzed with over 5-times higher catalytic efficiency than the best γ-lactone (nonanoic-γ-lactone (Fig. 2XII), 2.04 ± 1.12 × 10^5 M^-1 s^-1). The latter is 2 times a better substrate than the best AHL substrate (3-oxo-C9-AHL (Fig. 2IV), 9.70 ± 0.18 × 10^4 M^-1 s^-1). Comparison of different lactones possessing different alkyl chain lengths confirmed the trend observed for AHLs, whereby acyl chains containing 7 carbons were preferred by the enzyme. For γ-lactones and δ-lactones, the alkyl chain length preferred by the enzyme is between 5 and 6 carbon atoms, and a similar specificity was observed for MCP, GqL and DIOEP enzymes [4,9,54]. Interestingly, whereas the short chain C4-AHL (Fig. S2I) is a poor substrate for SisLac, the γ-heptanolate lactone (Fig. S2II) (3 carbon atoms in the alkyl side-chain) shows a 10^5 higher catalytic efficiency. In fact, lactones with very short or without side-chains (dihydrocoumarin (Fig. S2VII), δ-butyrolactone (Fig. S2V), δ-valerolactone (Fig. S2XI), ε-caprolactone (Fig. S2XVI)) constitute better substrates than C4-AHL (Fig. S2I).

Structural Analysis of SisLac

SisLac is homodimeric in the crystal structure with overall dimensions of the monomers of being approximately 39 × 40 × 36 Å. As for its close homolog SsoPox [13] and related PLLs like DsOPH [5], GqL and GqL [9], SisLac is roughly globular and exhibits a (β/α)8 barrel topology. The active site consists of a binuclear center located at the C-Terminal of the barrel. Four histidines (His22, His24, His170, His199), one aspartic acid (Asp256) and a carboxylated lysine (residue 137) are coordinating the two metals. The two metal cations (possibly iron and cobalt, as seen for SsoPox [13] and OpdA [20]) are bridged by a water molecule that is presumed to be the catalytic nucleophile. The active site includes a long hydrophobic channel that was revealed by structural studies on SsoPox as the binding region of aliphatic chains for the AHL substrates [13]. Indeed, SisLac’s structure is overall very similar to the structure of SsoPox (root-mean-square deviation (r.m.s.d) for α-carbon atoms (over 314 atoms) of 0.35 Å).

Salt bridge network analysis. SisLac sequence exhibits approximately the same amino acid content as SsoPox, containing 14.3% (30%) of charged polar residues and 28.7% (28%) of charged residues. This is not surprising since both enzymes possess high sequence identity (91%) (see sequence alignment, Fig. 2). As described for SsoPox, the charged residues are mainly located at the protein surface, forming complex electrostatic networks [47] that includes 28 salt bridges implicating 46 residues. This charge network mainly differs by the substitution K14E in SisLac that suppress a salt bridge network between E12-K14-D15 of SsoPox.
and consequently creates a local concentration of 3 negative charges within 4 consecutive residues (Fig. 5A).

**Dimer interface analysis.** The dimer interface of SisLac comprises 46 residues (44 in SsoPox). The contacting area is almost identical and comprise a typical value for homodimers [42] (1770 Å² for SisLac structure, 1750 Å² for SsoPox structure, lower for other thermostable PLLs: 1728 Å² for GilL structure, 1692 Å² for GSP structure and 1473 Å² for DOPH structure). The nature of this interface is mainly hydrophobic in both enzymes, but SisLac’s interface tends to be more hydrophilic (56% for SisLac, 52.6% for SsoPox), more charged (SisLac 23.91%, SsoPox 20.45%) and less polar (SisLac 32.61%, SsoPox 36.36%). Four interface residues differ from SisLac to SsoPox but these substitutions do not fully explain the observed differences. Interestingly, the superposition of SisLac and SsoPox monomers shows a relative movement of the second SisLac monomer of about 5 Å relative to SsoPox’s, revealing that the interface area is slightly shifted (Fig. 5B). This reorganization of the dimer interface appears to be due to the substitution Q34Y, although it could also originate from the different crystal packing of both proteins. Whereas the two Q34 interact with each other’s in SsoPox structure, the bulkiness of both Y34 in SisLac imposes a reorientation of the dimer (Fig. 5C). This reorientation increases the monomers interpenetration, and makes one closer to the active site of the other. This trend was previously described while comparing the P. diminuta PTE and SsoPox structures [47], although these enzymes are far more divergent in sequence (about 30% identity). Although the biological importance and catalytic influence of dimerization for these enzymes remain unclear, second shell active site residues are notably involved in dimer formation.

### Key Substitutions between SisLac and SsoPox

The structural analysis revealed that the positions 14 and 34 seem to be the major impacting variations between SisLac and SsoPox structures, that may relate to dimerization changes (position 34) or stability (positions 14, 34). Mutational intermediates between SisLac and SsoPox have been constructed to evaluate the consequences of these substitutions (E14K and Y34Q) for the enzyme stability and activity. All the mutants exhibit lower Tm as compared to SisLac (102±2°C) and SsoPox (106°C) [6] while double variants (Y34Q-E14K) present the highest Tm among the variants (Table 7). The analysis of Tm reveals that the substitution Y34Q and E14K are destabilizing on SisLac background but the combination of both variations tends to restore partially the stability (Table 7). Additionally, the mutants have been characterized for catalytic activity against ethyl/methyl-paraoxon and for the best AHL, δ-lactone and γ-lactone substrates of set SisLac (Table 8). The efficiency of methyl-paraoxon hydrolysis is similar for set and the mutants, whereas the mutants exhibit higher catalytic efficiency against ethyl-paraoxon than the set enzyme. However, the mutants exhibit a dramatically reduced AHLase activity (Table 8). A similar trend is observed with δ/γ-lactones. These results clearly highlight the critical importance of these positions for SisLac stability and activity, and validate our structural analysis.

### Discussion

#### Catalytic Properties of SisLac

The pH dependence of SisLac was investigated and yields a bell-shaped curve with a pH optimum at pH 9, a consistent behavior with previously characterized PLLs [6,7] and PTEs [48]. This pH dependence profile is also in agreement with the commonly accepted hydrolysis mechanism where a water molecule activated

| Substrate | kcat (s⁻¹) | Km (μM) | kcat/Km (s⁻¹M⁻¹) |
|-----------|------------|---------|------------------|
| 3-oxo-C10-AHL (1V) | 10.65±0.16 | 111±18 | 9.63±(1.89) x 10⁴ |
| 3-oxo-C12-AHL (1VI) | 0.39±0.04 | 435±123 | 8.97±(3.45) x 10² |

r corresponds to racemic solution and f at the pure levorotatory enantiomer. Data obtained with cobalt as cofactor. Roman numeration corresponds to chemical structures of Fig. S2.

Table 5. AHL lactonase activity of SisLac.

Table 6. Oxo-lactone lactonase activity of SisLac.
SisLac lactonase Activity

A

B

C
by the bi-metallic active site serves as nucleophile [13]. Additionally, the metal dependence was assayed and the metal nature was found to modulate the catalytic activities, as previously described in PTESs [19] and PLLs [34]. Amongst the tested metals, SisLac shows preference for cobalt cations for both lactonase and paraoxonase activities (supplementary information & Fig. S6), as previously reported for the paraoxonase activity of SsoPox [6] and the lactonase activity of DoPHE [34], MCP [4] and GDL [9]. The metal dependence of SisLac may be related to the relative pKa of considered metal with H2O, since the pKa of Co2+/H2O is lower than that of Zn2+/H2O and Mn2+/H2O (8.9 versus 9.0, and 10.6, respectively [20]), thus Co2+ would better contribute to the activation of the nucleophile. In addition, Co2+ is more electronegative than Zn2+ and Mn2+ (1.86 versus 1.65 and 1.55, respectively [19]), thus being more efficient for stabilizing the developing negative charge on the transition state.

Phosphotriesterase Activity

The catalytic efficiency of paraoxon hydrolysis by SisLac at 25 °C (kcat/KM = 2.60(±0.58)×107 M⁻¹ s⁻¹) shows that SisLac is endowed with one of the highest paraoxonase activity amongst PLLs [3,4,5,6,8,9]. Moreover, this activity can be considerably increased by addition of 0.1% SDS (25 folds), suggesting that the enzyme has an interesting potential for catalytic improvement. The potential of this enzyme for organophosphorus compounds bio-decontamination is further illustrated by its ability to hydrolyze the nerve agent analog CMP-coumarin with significant efficiency (kcat/KM = 4.26(±1.86)×107 M⁻¹ s⁻¹).

Interestingly, we observed that methyl-paraoxon is a better substrate than ethyl-paraoxon, the same trend being monitored between methyl and ethyl-parathion. The fact that smaller substituents on the phosphorus center are preferred by the enzyme is consistent with the promiscuous nature of paraoxonase activity in SisLac. Moreover, the higher compaction of the SisLac monomer makes active sites closer to each other in the dimer as compared to SsoPox structure and thus could explain the substrate preference in disfavor of bulkier substrates.

In addition, SisLac exhibits very low catalytic efficiency towards P = S containing organophosphates (e.g. ethyl and methyl-parathion, malathion). The KM values are in the range of the native substrates (hundreds of μM), but the kcat values are extremely low (about 3 orders of magnitude lower than P = O containing OPs), which is a typical behavior for promiscuous activities [56]. PTESs, albeit preferring paraaxon to parathion as a substrate, do not exhibit such a pronounced thiono-effect [20,55]. PTESs constitutes a protein family that are believed to have diverged from PLLs like SisLac [3] in the last few decades to specifically hydrolyze man-made insecticides [57]. They thus may have evolved to suppress this thiono-effect in order to hydrolyze the most used pesticides (e.g. parathion).

Esterase and Lactonase Activity

PLLs have been previously characterized as poor esterases [3], and so is SisLac. Amongst the five tested esters, only nNP-acetate is a substrate for SisLac. The natural substrates of PLLs, lactones, being a specific class of esters, it is thus surprising that PLLs exhibit low esterase activity. It might results from a rather good binding to the active site (as suggested by observed KM for nNP-acetate), but with a large fraction of non-productive binding (very low kcat).

PLLs are natural lactonases that might be involved in quorum quenching [13]. Their precise substrates and biological function(s) are however still unknown. Lactones encompass two major families of compounds, the lipophilic aroma (oxo-lactones) and the Acyl Homoserine Lactones (AHLs) involved in quorum sensing. The quorum sensing is common in bacteria, but its existence in the archaeal world remains unclear, despite the finding of AHL-based quorum sensing stimulating molecules in Natronococcus occultus [58], the presence of biofilms in Sulfolobus sp. [59] and the recent characterization of complete carboxylated-AHLs quorum sensing system in methanogenic archaeon [60].

Our kinetic experiments show that SisLac prefers long aliphatic chain lactones, exhibiting optimal activity when the acyl chain contains 7 carbon atoms, as seen for SsoPox [52]. SisLac show also preference for 3-oxo-AHLs and hydrolyzes poorly short chain AHLs. It is interesting to notice that other PLLs, like AhiA and PPH, hydrolyze short and long chain lactones with similar catalytic efficiency [3]. It is thus possible that within the PLL family, different sub-groups of enzymes exhibit different specificities, and thus possibly different physiological functions. In addition, we show that SisLac is a proficient enzyme against oxo-lactones (best substrate: undecanoic-δ-lactone, kcat/KM = 1.77(±0.04)×109 M⁻¹ s⁻¹), and hydrolyzes more efficiently long chain lactones, with a preference for 5 to 6 acyl chain carbon atoms. This preference is similar to that observed for AHLs as substrates, and possibly indicates that oxo-lactones and AHLs acyl chains bind into a similar pocket, most likely the hydrophobic channel connected to the active site that was depicted for SsoPox structure [13,47]. However, interestingly, whereas the short chain C4-AHL is a poor substrate for SisLac, the heptanoic δ-lactone (3 carbon atoms in the acyl chain) shows a 104 higher hydrolysis efficiency. In addition, lactones with very short or without acyl chains (dihydrocoumarin, γ-butyrolactone, δ-valerolactone, ε-caprolactone) constitute better substrates than C4-AHL. Altogether, these features might reveal that γ- and δ-lactones utilize an alternate binding mode for the lactone ring than AHLs, and/or that C4-AHL does not bind in a catalytically relevant fashion to SisLac.

Table 7. Melting temperature of SisLac and its variants.

| Protein   | Tm (°C) |
|-----------|---------|
| SisLac wt  | 102±2   |
| SisLac E14K | 96±1   |
| SisLac Y34Q | 94±2   |
| SisLac E14K-Y34Q | 98±2   |

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Structural Determinants for Thermal Stability

The major structural determinants explaining the high thermal stability of hPLIs have been documented with the example of \textit{SsoPox} [47], the comparison with \textit{GoP} and \textit{DOPH} enzymes [8], and are part of the classical properties described for hyperthermostable proteins [61]. The structures of these enzymes, including \textit{SisLac}, exhibit a high number of salt bridges organized in complex networks of charges at the protein surface that may rigidify the global protein architecture. Moreover, the homodimer interface is larger and more hydrophobic (see Results) and the overall structure is more compact than mesophilic counterparts [47]. Here we observed that the interface area between \textit{SisLac}'s monomers is slightly shifted, as compared with \textit{SsoPox} structure. This reorganization of the dimer interface is consistent with observations made in solution. Indeed, as observed in crystals, both enzymes are dimeric at 25 °C. The importance of hydrophobic contacts within the dimer interface of \textit{SisLac} and \textit{SsoPox} explain the dimerization of the proteins. Since the hydrophobic effect increases with temperature, it is highly probable that these enzymes could be dimers at physiological temperatures (50-90 °C) [2,49].

Interestingly, both \textit{Sulfobolus} species from which \textit{SisLac} and \textit{SsoPox} enzymes originates lives in similarly extreme environments (\textit{S. solfitarius} from 50 to 87 °C [49], \textit{S. islandicus} from 59 to 91 °C [2]) and exhibit similar thermostability (\(T_m = 102 \pm 2^\circ\mathrm{C}\) for \textit{SisLac} and \(T_m = 106^\circ\mathrm{C}\) for \textit{SsoPox} [6]). Taking advantage from their high sequence identity between the two proteins, we studied the substitution K14E in \textit{SisLac} (as compared to \textit{SsoPox}) that breaks a salt bridge network at the C-terminus of the protein, a region concentrating the highest divergences among hPLIs as revealed by sequence alignment (\textit{i.e. SsoPox, SisPox and SisLac}). E14 engenders in \textit{SisLac} a cluster of 3 negatively charged residues at the surface of the structure allowing to evaluate the contribution of these electrostatic interactions to the enzyme stability and activity. Moreover, another key substitution occurred between the two enzymes in the homodimerization interface. Q34Y is indeed a key substitution in the interface since it consists in a “pivot” residue, \textit{i.e.} a residue that contacts its equivalent in the second protein molecule while forming the dimer, and seems to be responsible for the observed dimerization shift between \textit{SisLac} and \textit{SsoPox} structures. Q34Y is, moreover, the substitution between \textit{SisLac} and \textit{SsoPox} that is the closest in space to the active site (second shell). We therefore studied the effects of this variation on \textit{SisLac}'s activity and stability.

Surprisingly, whereas K14 in \textit{SsoPox} is involved in a large network of charged interactions and may contribute to the overall protein rigidity [47], the variation E14K in \textit{SisLac} is destabilizing (\(T_m\) is decreased by 6 °C). The variation of the pivot interface residue Y34Q is also destabilizing on \textit{SisLac} background (decrease of \(T_m\) by 8 °C). Interestingly, the double variant E14K-Y34Q that carries two destabilizing mutations exhibits a higher \(T_m\) than the single variants, revealing the highly epistatic nature of these positions. Moreover, whereas the promiscuous phosphotriesterase activity is not altered by these substitutions, the lactonase activity, especially the AHLase activity is considerably reduced (by \(-100\text{ folds}\) as compared to \textit{wt}). The influence of E14K on \textit{SisLac}'s catalytic activity is not obvious from a structural analysis. However, position 34 comprises a second shell residue, and the overall dimer interface is in the vicinity of the active site. Mutation of position 34 highly influences the protein dimerization and thus the degree of monomers interpenetration. Monomer active sites being close one to each other, their interpenetration could influence substrate specificities and catalytic efficiencies by fine steric or dynamic constraints which can’t be evaluated by

### Table 8. Kinetic characterization of mutational intermediates between \textit{SisLac} and \textit{SsoPox}.

| Substrate | Protein | \(k_{cat}\) (s\(^{-1}\)) | \(K_m\) (\(\mu\)M) | \(k_{cat}/K_m\) (M\(^{-1}\)s\(^{-1}\)) |
|-----------|---------|-----------------|-----------------|-----------------|
| Ethyl Paraaxon | \textit{SisLac} wt | 1.42(0.09) | 5439(873) | 2.60(0.58) x 10\(^2\) |
| | \textit{SisLac} E14K | 0.14(0.01) | 30(5) | 4.50(0.60) x 10\(^3\) |
| | \textit{SisLac} Y34Q | 0.13(0.001) | 150(12) | 8.66(0.89) x 10\(^2\) |
| | \textit{SisLac} E14K-Y34Q | 0.17(0.01) | 132(19) | 1.28(0.22) x 10\(^3\) |
| Methyl Paraaxon | \textit{SisLac} wt | 7.40(1.26) | 1739(417) | 4.26(1.74) x 10\(^3\) |
| | \textit{SisLac} E14K | 0.30(0.01) | 50(6) | 6.00(0.80) x 10\(^3\) |
| | \textit{SisLac} Y34Q | 0.42(0.01) | 261(24) | 1.61(0.19) x 10\(^3\) |
| | \textit{SisLac} E14K-Y34Q* | 0.86(0.21) | 620(198) | 1.39(0.78) x 10\(^3\) |
| 3-oxo-C6-AHL (\(\mu\)) | \textit{SisLac} wt | 4.1(0.09) | 42(7) | 9.70(1.84) x 10\(^4\) |
| | \textit{SisLac} E14K | 0.85(0.06) | 917(150) | 9.27(2.17) x 10\(^3\) |
| | \textit{SisLac} Y34Q | 0.92(0.05) | 1214(135) | 7.58(1.25) x 10\(^3\) |
| | \textit{SisLac} E14K-Y34Q | 0.97(0.04) | 1017(98) | 9.54(1.31) x 10\(^3\) |
| nonanoic-\(\gamma\)-lactone (\(\mu\)) | \textit{SisLac} wt | 3.10(0.13) | 15(8) | 2.04(1.12) x 10\(^5\) |
| | \textit{SisLac} E14K | 1.88(0.07) | 25(10) | 7.52(3.29) x 10\(^4\) |
| | \textit{SisLac} Y34Q | 1.91(0.05) | 61(9) | 3.13(0.17) x 10\(^4\) |
| | \textit{SisLac} E14K-Y34Q | 1.99(0.12) | 47(18) | 4.23(1.87) x 10\(^4\) |
| Undecanoic-\(\omega\)-lactone (\(\mu\)) | \textit{SisLac} wt | 17.65(0.38) | <10 | >1.77(0.04) x 10\(^6\) |
| | \textit{SisLac} E14K | 14.09(0.59) | 42(16) | 3.35(1.41) x 10\(^5\) |
| | \textit{SisLac} Y34Q | 12.80(0.51) | 121(22) | 1.06(0.23) x 10\(^5\) |
| | \textit{SisLac} E14K-Y34Q | 12.91(0.58) | 43(13) | 3.00(1.04) x 10\(^5\) |

Data obtained with cobalt as cofactor. * Hydrolysis of methyl-paraaxon by \textit{SisLac} E14K-Y34Q exhibits a substrate inhibition profile with \(K_i = 855 \pm 376 \mu\)M.

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structural analysis. These identified key substitutions, however, does not fully explain the observed different catalytic properties of SisLac and SisPox. The active site residues and configuration of these two enzymes being similar, these discrepancies might be partly mediated by yet unidentified substitutions distant from the active site.

Reconstructed mutational intermediates between SisLac and SisPox have lower fitness both in term of stability and AHLase activity. Despite the very high sequence identity between both proteins (91%), it may indicate that the evolutionary route that links them already comprise a fraction of highly epistatic mutations. In other words, the mutations that accumulate at a very early stage of divergence might not only be neutral but a fraction of them are highly cooperative.

Supporting Information

Figure S1 Chemical structure of phosphotriesters (I-VI) and esters (VII-XI).

Figure S2 Chemical structure of AHLs (I-VI), γ-lactones (VII-XI), δ-lactones (XII-XV) and other lactones (XVI-XVII).

Figure S3 Electronic density map of SisLac at 2.7 Å resolution.

Figure S4 Sequence alignment of PLLs from Sulfolobus species.

Figure S5 Thermostatability analysis of SisLac by circular dichroism.

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Figure S6 SisLac metal preference.

Information S1 Supplementary information for SisLac metal preferences.

Table S1 Primers used for site directed mutagenesis.

Table S2 Kinetics protocols.

Table S3 Ethyl-paraoxonase comparison between SsoPox, SacPox and SisLac.

Table S4 Phosphotriesterase activity comparison between GsP, DrOPH and SsoPox and SisLac.

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Accession Number

The coordinate file and the structure factors file of the SisLac structure have been deposited to the Protein Data Bank under the accession number 4G2D.

Author Contributions

Conceived and designed the experiments: ME JH EC. Performed the experiments: ME JH GG. Analyzed the data: ME JH EC. Wrote the paper: ME JH.
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