SacPox from the thermoacidophilic crenarchaeon
*Sulfolobus acidocaldarius* is a proficient lactonase
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**Abstract**

**Background:** SacPox, an enzyme from the extremophilic crenarchaeal *Sulfolobus acidocaldarius* (*Sac*), was isolated by virtue of its phosphotriesterase (or paraoxonase; Pox) activity, i.e. its ability to hydrolyze the neurotoxic organophosphorus insecticides. Later on, SacPox was shown to belong to the Phosphotriesterase-Like Lactonase family that comprises natural lactonases, possibly involved in quorum sensing, and endowed with promiscuous, phosphotriesterase activity.

**Results:** Here, we present a comprehensive and broad enzymatic characterization of the natural lactonase and promiscuous organophosphorus hydrolase activities of SacPox, as well as a structural analysis using a model.

**Conclusion:** Kinetic experiments show that SacPox is a proficient lactonase, including at room temperature. Moreover, we discuss the observed differences in substrate specificity between SacPox and its closest homologues SsoPox and SisLac together with the possible structural causes for these observations.

**Keywords:** Lactonase, PLL, Quorum sensing, Phosphotriesterase, Extremophile, Thermoacidophile

**Background**

Phosphotriesterase-Like Lactonases (PLLs) are natural lactonases (EC 3.1.1.25) (Figure 1C, D, E) with promiscuous phosphotriesterase activity (EC 3.1.8.1) (Figure 1A) [1,2]. They are structurally closely related to bacterial phosphotriesterases (PTEs) [3-6], such as *Brevundimonas diminuta* PTE (BdPTE; ~30% sequence identity) [7]. PTEs naturally hydrolyze neurotoxic organophosphorus (OPs) compounds (Figure 1A) such as paraoxon (the active metabolite of the insecticide parathion) with catalytic constants that approach the diffusion limit (i.e. $k_{cat}/K_M \sim 10^{8} \text{M}^{-1} \text{s}^{-1}$) [7]. Because OPs have been massively used as pesticides since the 50’s [8], PTEs are believed to have emerged in few decades from a PLL progenitor [2], providing a new source of phosphorus to bacteria, and consequently a selective advantage [8].

Both enzyme families exhibit the same ($\beta_8$α$_8$)-barrel topology [9,10] and belong to the amidohydrolase superfamily [11,12]. Their structure consists of 8 $\beta$-strands forming a central barrel surrounded by 8 $\alpha$-helices. The active site is constituted by a bimetallic center (two metal cations) localized at the C-terminus of the barrel. Metal cations are coordinated by four histidines, an aspartic acid and a carboxylated lysine residue [9]. While the nature of the bimetallic center can vary depending on the enzyme nature and the purification procedure [3,5,13,14], the catalytic mechanism is presumed to be identical. The bimetallic center activates a water molecule into a hydroxide ion which performs a nucleophilic attack onto the electrophilic center [9,15].

The difference in substrate specificities of PLLs and PTEs seems mainly governed by variation in the connecting loops of the barrel [2,16]. Major differences between PTEs and PLLs reside in the active site loop size and conformation [1,2]. Indeed, loop 7 is shorter in PLLs than in PTEs whereas the loop 8 is larger, forming a hydrophobic channel that accommodates lactones aliphatic chain [9]. Loop 7/8 length and sequence also differ within the PLL family and led to the identification of two different subfamilies: PLLs-A and PLLs-B [2]. Both subfamilies exhibit different substrate specificities: PLLs-B are exclusively o xo-lactonases (Figure 1DE) whereas PLLs-A hydrolyze efficiently o xo-lactones and Acyl-Homoserine Lactones (AHLs, Figure 1C) [2]. AHLs are messenger molecules involved in a bacterial communication system.

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dubbed *quorum* sensing (QS) [17]. QS regulates the expression of numerous genes, and enables bacterial population to adopt a “group” behavior, including the expression of virulence factors of some pathogens [18,19]. The involvement of PLLs-A in *quorum* sensing has not yet been demonstrated, and these enzymes are often found with no other AHL components, including in archaeal species [20]. However, the fact that they hydrolyze specifically the natural enantiomer of AHL indicates that it may be their native substrate [16].

PLLs are promiscuous enzymes that catalyze two chemical reactions of potential biotechnological interest. Indeed, the inhibition or “quenching” of the QS is seen as a possibly promising strategy to develop innovative therapies [21-25]. Indeed, lactonases such as PLLs can inhibit QS (known as *quorum* quenching, i.e. QQ) [26,27] and thereby annihilate the virulence of micro-organisms possessing an AHL-based QS system [28]. Moreover, PLLs are endowed with relatively low phosphotriesterase activity, but might be optimized against OPs and subsequently used for degrading organophosphorus pesticides [3,5,6,9,29] and nerve agents [30], for which no satisfactory remediation methods are currently available [31].

In addition, several PLLs members are thermostable [3,4,6,32-34]; e.g. PLLs from extremophilic crenarchaeon sources [3,4,16,34]. These counterparts exhibit industry-compatible properties (e.g. thermal and detergent resistance) [35-37]; making them good starting point for in vitro improvement protocols [37,38]. Several studies report the engineering of thermostable PLLs and improvement of catalytic efficiency against OPs, including for *SsoPox* [16,39], *DrOPH* (*Deinococcus radiodurans* organophosphorus hydrolase) [6,40] and GKL (*Geobacillus kaustropilus* lactonase) [41] but also for the lactonase activity of *SsoPox* [16], MCP (*Mycobacterium avium* subsp. *Paratuberculosis* K-10 lactonase) [42] and GKL [43].

Here we focus on *SacPox*, the PLL from the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* (living conditions: 55–85°C, pH 2–3) [44]. *SacPox* was originally isolated and studied for its ability to hydrolyze OP compounds at high temperature [4]. The enzyme shares about 30% of sequence identity with *BdPTE* and

![Figure 1 Chemical structure of *SacPox* substrates.](image-url)
about 70% with its closest homologues, i.e. SsoPox from *Sulfolobus solfataricus* [3,45]. Being an enzyme from a hyperthermophile, SacPox is however less stable than SsoPox (half-life of 5 min at 90°C [4] and of 4 h at 95°C [3,46], respectively). The kinetic characterizations performed on SacPox revealed that it hydrolyzes OP, ester and lactone molecules at high temperature [4,13]. However, only few substrates have been tested, and no natural lactones were assayed as substrate. In this study, we performed a broad kinetic characterization of SacPox at room temperature (25°C) for several OPs, esters (Figure 1B) and lactone molecules including AHLs, γ-lactones and δ-lactones in the aim to evaluate the biotechnological potentialities of this enzyme.

**Methods**

**Sequence alignment**

The sequence alignment was performed based on the previously published PLL sequence alignment [2], using the *T-coffee* server (expresso) [47,48] and manually improved with the *seaview* software [49]. It contains 29 different sequences (Additional file 1: Table S1). The sequence alignment was represented using the *BioEdit* 7.1.3 software [50]. Protein sequence identities were computed using *ClustalW* server [51]. The phylogenetic tree was performed using *PhyML* [49] and default parameters.

**Protein production and purification**

The protein production and subsequent purification steps were performed analogously to previously described [16,33,34,45,52-54]. In brief, the protein was heterologously produced in *Escherichia coli* strain BL21 (DE3)-pGro7/GroEL (TaKaRa) at 37°C in ZYP medium [55]. When OD600nm reaches 0.8, protein production was induced with addition of arabinose (0.2%, w/v) and CoCl2 (2 mM) and temperature transition to 25°C for 20 hours. Cells were harvested by centrifugation, and pelleted cells were suspended in *lysis buffer* (50 mM HEPES pH 8, 150 mM NaCl, 0.2 mM CoCl2, lysozyme 25 mg/ml, PMSF 0.1 mM, DNase I 10 mg/ml), stored at −80°C during 2 hours; then sonicated 3 times during 30 seconds (Branson Sonifier 450, 80% intensity and microtype limit of 8) and centrifuged. Taking advantage of the high stability of SacPox, the supernatant was heated at 70°C during 30 minutes and centrifuged before proceeding a STREP-TRAP affinity chromatography step (GE Healthcare, Uppsala, Sweden). The sample was then cleaved by the Tobacco Etch Virus protease (TEV, ratio 1:20, w/w [56]) during 20 hours at 30°C prior to be loaded a second time on STREP-TRAP affinity chromatography. The flow through containing the cleaved protein was then concentrated and loaded on a size exclusion column (S75-16-60; GE Healthcare, Uppsala, Sweden). The protein purity and identity were checked by SDS-PAGE and mass spectrometry analysis (MS platform Timone, Marseille, France). The protein concentration was determined using a nanospectrophotometer (Nanodrop, Thermofisher Scientific, France) using its molar extinction coefficient (SacPox ε280 nm = 35 307.7 M⁻¹ cm⁻¹) calculated by the *PROT-PARAM* server [57].

**Kinetic characterization**

**General procedures**

Catalytic parameters were evaluated at 25°C and recorded with a microplate reader (Synergy HT, BioTek, USA) and the Gen5.1 software as previously explained [16,33,52,54]. The reaction was performed in a 200 μl volume using a 96-well plate with a 6.2 mm path length as previously described [33]. The collected data were subsequently fitted to the Michaelis-Menten (MM) equation [58] using *GraphPad Prism 5.00* (GraphPad Software, San Diego California USA, www.graphpad.com). In cases where Vmax could not be reached, the catalytic efficiency was obtained by fitting the linear part of MM plot to a linear regression using *GraphPad Prism 5.00* software.

**OP hydrolase and esterase kinetics**

Standard assays for organophosphates (Figure 1A) and esters (Figure 1B) were performed in *activity buffer* (50 mM HEPES pH 8, 150 mM NaCl, 0.2 mM CoCl2) by measuring the p-nitrophenolate release over time at 405 nm (ε405 nm = 17 000 M⁻¹ cm⁻¹). For ethyl-paraoxon (Additional file 1: Figure S1V) hydrolysis was followed at 412 nm in *activity buffer* added of 2 mM DTNB to follow the release of free thiols (ε412 nm = 13 700 M⁻¹ cm⁻¹). The time course hydrolysis of dihydroumarin (Additional file 1: Figure S1X), CMP-coumarin (Additional file 1: Figure S1VI) and phenylacetate (Additional file 1: Figure S1VII) were respectively monitored at 270 nm (ε270 nm = 1 400 M⁻¹ cm⁻¹), 412 nm (ε412 nm = 37 000 M⁻¹ cm⁻¹) and 270 nm (ε270 nm = 1 400 M⁻¹ cm⁻¹).

**Lactonase kinetics**

Kinetics monitoring the lactone hydrolysis were performed according to a previously described protocol [33]. The lactone hydrolysis was monitored in the *lactonase buffer* (2.5 mM Bicine pH 8.3, 150 mM NaCl, 0.2 mM CoCl2, 0.25 mM Cresol purple and 0.5% DMSO) with different AHLs (Figure 1C) i.e. C4-AHL (r), C6-AHL (r), C8-AHL (r), 3-oxo-C8-AHL (l), 3-oxo-C10-AHL (l) (Additional file 1: Figure S1XI-XVI) and oxo-lactones (Figure 1D,E) i.e. ε-caprolactone, γ-heptanolide (r), Nonanoic-γ-lactone (r), Nonanoic-δ-lactone (r), Undecanoic-γ-lactone (r), Undecanoic-δ-
Figure 2 (See legend on next page.)
lactone (r), Dodecanoyl-γ-lactone (r) and Dodecanoyl-δ-lactone (r) [Additional file 1: Figure S1XVII-XXIV]. Cresol purple (pK_a 8.3 at 25°C) is a pH indicator (577 nm) used to monitor the acidification of the medium following lactone ring hydrolysis ($E_{577nm} = 5 \times 500 \text{ M}^{-1} \text{ cm}^{-1}$).

**Structural modeling and structural analysis**

The SacPox structure was modelled using the *EsyPred3D* server using SacPox protein sequence as query and SsoPox structure (2VC5) as template [59]. Structures were analyzed and figure made using PyMol [60].

**Results**

First classified within the bacterial PTEs, SacPox shares in fact only 33.8% sequence identity with *BdPTE* (Additional file 1: Table S2). SacPox indeed belongs to the PLLs-A (Figure 2A) [2]: it shares 76.1% of sequence identity with its closest homologues SsoPox and SisLac, and only 30.6% identity with the PLL-B DrOPH. Together with SisLac and SsoPox, SacPox comprises the crenarchael clade of the PLLs-A (Figure 2A). The sequence alignment highlights the strict conservation of essential active site residues between the different clades (Figure 2B).

**Enzymatic characterization**

**Phosphotriesterase activity**

SacPox ability to hydrolyze insecticides ethyl/methyl-paraoxon, ethyl/methyl-parathion and malathion has been evaluated (Table 1). The best SacPox phosphotriester substrate, methyl-paraoxon is processed with moderate catalytic efficiency ($k_{cat}/K_M = 1.10(\pm0.17) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$), low rate ($k_{cat} = 0.307 \text{ s}^{-1}$) and low $K_M (278.3 \mu\text{M})$. Very similar catalytic efficiencies were recorded for SsoPox and SisLac: $k_{cat}/K_M$ of 1.27×10^3 M^{-1}s^{-1} and 4.26×10^3 M^{-1}s^{-1}, respectively [33,52]. Ethyl-paraoxon comprise a slower substrate, ($k_{cat}/K_M = 2.81\times10^2 \text{ M}^{-1} \text{ s}^{-1}$), highlighting the enzyme preference for OP substrates with small substituents. No hydrolysis could be measured for ethyl-parathion and malathion, whereas a low catalytic efficiency was recorded for methyl-parathion ($k_{cat}/K_M = 4.31 \text{ M}^{-1} \text{ s}^{-1}$). This specificit profile illustrates the clear preference of SacPox for oxono-phosphotriesters rather than thiono-phosphotriesters; as previously observed for SsoPox [52] and SisLac [33]. Moreover, whereas anionic detergents like SDS can significantly stimulate SsoPox phosphotriesterase activity [52], the same treatment on SacPox yields only a 2-fold increase in catalytic efficiency with ethyl-paraoxon as substrate. Finally, we show that SacPox hydrolyzes CMP-coumarin ($k_{cat}/K_M = 4.38 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$), albeit with 20-fold lower catalytic efficiency than SsoPox [52].

**Esterase activity**

The ability of SacPox to hydrolyze phenyl-acetate, pNP-acetate and pNP-decanoate (Additional file 1: Figure S1VII-IX) has been evaluated (Table 2). While no activity could be detected against pNP-decanoate, SacPox exhibits low catalytic efficiencies against both phenyl-acetate and pNP-acetate ($k_{cat}/K_M = 50 \text{ M}^{-1} \text{ s}^{-1}$). This weak activity against classical esters differs from previous studies on the close homologues SsoPox and SisLac, for which activity has only been recorded on pNP-acetate [33].

**Lactonase activity**

The catalytic parameters of SacPox for various lactone substrates have been measured, including against oxo-lactones (lipophilic aroma), AHLs and dihydrocoumarin (Table 3). Our results indicate a preference of SacPox for o xo-lactone substrates; i.e. γ-heptanolid and nonanoic-γ-lactone ($k_{cat}/K_M = 2.5\times10^3 \text{ M}^{-1} \text{ s}^{-1}$), while AHLs are about 10 times worse substrates (i.e.; C8 AHLs, $k_{cat}/K_M = 5\times10^3 \text{ M}^{-1} \text{ s}^{-1}$). Furthermore, it seems that SacPox prefers AHLs vs 3-oxo-AHLs since the $K_M$ for C8 aliphatic chains is 5-fold lower than that for 3-oxo-C8 AHLs. Overall, long aliphatic chain substrates AHLs are better substrates for the enzyme. Indeed, short aliphatic chain AHLs are not hydrolyzed by SacPox. Interestingly, this preference is not retained for oxo-lactones, for which molecules with short

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**Table 1 Phosphotriesterase kinetic parameters**

| Substrate       | $k_{cat}$ (s^{-1}) | $K_M$ (μM) | $k_{cat}/K_M$ (M^{-1}s^{-1}) |
|-----------------|--------------------|------------|-----------------------------|
| Paraoxon        | 0.12 ± 0.01        | 434 ± 54   | 2.81 (±0.38) × 10^2          |
| Paraoxon 0.01% SDS | 0.28 ± 0.01        | 537 ± 48   | 5.22 (±0.51) × 10^2          |
| Paraoxon 0.1% SDS | 0.25 ± 0.01        | 405 ± 21   | 6.10 (±0.34) × 10^2          |
| Methyl Paraoxon | 0.31 ± 0.02        | 278 ± 40   | 1.10 (±0.17) × 10^3          |
| Parathion       | ND                 | ND         | ND                          |
| Methyl Parathion| ND                 | ND         | 4.31 ± 0.20                 |
| Malathion       | ND                 | ND         | ND                          |
| CMP-Coumarin    | 0.28 ± 0.02        | 642 ± 89   | 4.38 (±0.68) × 10^2          |

ND correspond to Not Detected hydrolysis. Results have been obtained with cobalt as cofactor.
Table 2 Esterase kinetic parameters

| Compound     | k_{cat} (s^{-1}) | K_M (μM) | k_{cat}/K_M (M^{-1}.s^{-1}) |
|--------------|------------------|----------|----------------------------|
| Phenyl-acetate| 0.35 ± 0.05      | 8 181 ± 1750 | 423 ± 11.1                |
| pNP-acetate   | 0.13 ± 0.01      | 2 107 ± 313   | 60.1 ± 9.9                |
| pNP-decanoate | ND               | ND        | ND                        |

ND correspond to Not Detected hydrolysis. Results have been obtained with cobalt as cofactor.

or without aliphatic chain are efficiently hydrolyzed (k_{cat}/K_M = 10^4 M^{-1}.s^{-1}). As previously observed for SsoPox and SisLac [16,33], this feature may reveal a potential alternative binding mode of these compounds in SapoX active site. Finally, contrary to SsoPox and SisLac [16,33], SapoPox does not hydrolyze dihydrocoumarin.

Structural analysis

Numerous attempts to crystallize SapoPox were made, with no success (Elias, Hiblot, Gotthard & Chabriere, unpublished). A previous structural model was generated by homology modeling based on BdpTE structure [4] (~33.8% sequence identity with SapoPox), but yielded little insights given the moderate sequence identity with the template and the very significant differences in the active site loops between these two representatives of distinct enzyme families [19,16]. Here we generated a homology-based model using the structure of SsoPox as template (76.1% of sequence identity; Additional file 1: Table S2).

As expected, the SapoPox model structure almost perfectly superimposes to the SapoPox crystal structure (Figure 3A). Residues forming the active site are all conserved and residues involved in loops 7 and 8 occupy nearly identical conformation in SapoPox and SsoPox but also in SisLac structures (Figure 3B). Noteworthy, loop 8 is partially structured into an a-helix, as seen in X-ray structures of SsoPox and SisLac. A substitution (L266 in SacPox; T265 in SsoPox and SisLac) in loop 8 may slightly alter the shape of the aliphatic channel. But overall, the active site of SacPox and SsoPox are nearly identical (Figure 2B). Furthermore, four other substitutions between SapoPox and its close homologues can be seen in loop 8: SapoPox exhibits a K at position 268, instead of an R residue (R267 in SisLac), Y271 instead of L (L270 in SisLac), K278 instead of R (R277 in both SisLac and SapoPox), and M281 instead of I (I280 in SapoPox) (Additional file 1: Figure S2). While the structural model suggests that these substitutions are not affecting directly the binding cleft of SapoPox, they might modulate loop 8 conformation and its dynamics. Indeed, it was shown in the close homologue SsoPox that a single substitution in loop 8 (W263 in SapoPox, equivalent to W264 in SapoPox) increases the conformational flexibility of loop 8, thereby conferring higher promiscuity to the enzyme [16]. The effect is in fact so dramatic that the substitution in SapoPox of W263 by any of the 19 other natural amino acids yields a variant with improved phosphotriesterase activity [16]. Additionally, loop 8 being involved in the accommodation of the aliphatic substituent of lactones substrates [9], mutations in this loop can also affect the lactonase activity [16].

Discussion

Here we show that SapoPox is a proficient lactonase (~10^4 M^{-1}.s^{-1}) and can hydrolyze both oxo-lactones and AHLs. Nevertheless, SapoPox have a slightly different substrate specificity than its close homologues [16,33]. Indeed, SapoPox exhibits slightly lower catalytic efficiencies, prefers AHLs over 3-oxo-AHLs and does not show any activity against dihydrocoumarin. Interestingly, as noted for SisLac and SapoPox [16,33], SapoPox clearly prefers long chain AHLs, but can efficiently hydrolyze short chain or oxo-lactones without aliphatic substituents. This feature could reflect a putatively different binding mode of AHLs and oxo-lactones into PLLs active sites. We note that the biological role of lactonases such as PLLs is yet unclear, especially in extremophilic archaea where no AHL-based quorum sensing systems have been identified so far.

SapoPox also exhibits promiscuous esterase and phosphotriesterase activities, a common feature of PLLs. Similarly to SapoPox and SisLac [33,52], SapoPox prefers OPs with small substituents. Moreover, SapoPox also shows a clear preference for oxono-phosphotriesters, rather than thiono-phosphotriesters, a feature previously dubbed thino-effect [52]. Interestingly, SapoPox, SisLac and SapoPox exhibit similar catalytic efficiencies against OPs (10^{2−3} M^{-1}.s^{-1}) at 25°C, efficiencies that are close to those measured at much higher temperatures [4].

Table 3 Lactonase kinetic parameters

| Compound       | k_{cat} (s^{-1}) | K_M (μM) | k_{cat}/K_M (M^{-1}.s^{-1}) |
|----------------|------------------|----------|----------------------------|
| C4 AHL         | ND               | ND       | ND                        |
| C6 AHL         | ND               | ND       | ND                        |
| C8 AHL         | 0.94 ± 0.02      | 178 ± 26 | 5.28 (±0.77) × 10^3        |
| 3-oxo C6 AHL   | ND               | ND       | ND                        |
| 3-oxo C8 AHL   | 0.89 ± 0.07      | 836 ± 178| 1.07 (±0.25) × 10^3        |
| 3-oxo C10 AHL  | 1.03 ± 0.04      | 213 ± 33 | 4.88 (±0.77) × 10^3        |
| γ heptanolide  | 10.25 ± 0.50     | 388 ± 62 | 2.64 (±0.44) × 10^4        |
| Nonanoic-γ-lactone | 2.64 ± 0.07   | 109 ± 19 | 2.44 (±0.44) × 10^4        |
| Undecanoic-γ-lactone | 0.34 ± 0.01   | 578 ± 78 | 5.89 (±0.54) × 10^2        |
| Dodcenoic-γ-lactone | 0.53 ± 0.03  | 242 ± 60 | 2.21 (±0.57) × 10^3        |
| Nonanoic-δ-lactone | 4.55 ± 0.21   | 348 ± 53 | 1.31 (±0.21) × 10^4        |
| Undecanoic-δ-lactone | 1.05 ± 0.05  | 168 ± 37 | 6.22 (±1.40) × 10^3        |
| Dodecanoic-δ-lactone | 3.34 ± 0.07   | 185 ± 27 | 1.81 (±0.27) × 10^4        |
| ε caprolactone | 15.04 ± 0.47     | 1 031 ± 83| 1.46 (±0.13) × 10^4        |

ND correspond to Not Detected hydrolysis. Results have been obtained with cobalt as cofactor.
The structural model shows that SacPox structure is very close to that of SsoPox (Figure 2A). Most critically, the active sites of both enzymes are essentially identical (Figure 2B), with the exception of position 266 (I in SacPox, T in SsoPox and SisLac). This substitution might partly account for the observed differences in substrates specificity between these enzymes, and would thereby represent an interesting target for future mutagenesis studies. But four other substitutions in loop 8 between these close homologues might be involved as well, and comprise also interesting options for mutagenesis studies (K268R, Y27IL, K278R and M281I). A recent study on SsoPox highlighted how profound the effect on catalysis of a single substitution on loop 8 (W263) can be [16]. Therefore, substitution T266I, and/or the four others on loop 8, might contribute to the observed differences between SacPox and SsoPox in substrate specificity, in combination with other factors that cannot be assessed.

**Figure 3 Structural model of SacPox.**

A. Structural superposition of SsoPox structure (2VC5; grey) and the SacPox model (green). Cobalt, iron and the catalytic water molecule are respectively represented by pink, orange and red spheres. Bimetallic center coordinating residues are represented as sticks. B. Active site view of superimposed SsoPox structure (grey) and the SacPox model (green). Several active site residues are represented as sticks. Numbering is made according to SacPox sequence.
by a structural model such as subtle changes in active site loops conformation and dynamics [16,33]. Indeed, the observed differences in the detergent stimulation between both enzymes (SacPox is only weakly stimulated by SDS, as compared to SsoPox) could well be a manifestation of different dynamics of their respective active site loops.

Conclusions
To conclude, we here demonstrate that albeit being initially isolated, characterized, and named after its ability to degrade the insecticide paraoxon (pox; [4]), SacPox is putatively a native lactonase, capable of hydrolyzing these compounds with significant catalytic efficiencies at 25°C (up to 10^4 M⁻¹s⁻¹). The extensive kinetic characterization reveals some substrate specificity differences between SacPox and its close homologues SisLac and SsoPox, and the proposed structural model of SacPox suggests putative candidates (e.g. I266) that could account for these observations. Such positions might constitute interesting targets for future engineering studies, with the aim of improving or altering the catalytic properties of SacPox.

Additional file

Additional file 1: Figure S1. Chemical structure of phosphoesters (I-VII), esters (VIII-XX) and lactones (X-XXIV). Figure S2. Superposition of SsoPox, SisLac and SacPox structural models. Table S1. Accession numbers of the sequences used in the phylogeny study. Table S2. Sequence identity matrix.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
JH, GG and ME planned the experiments. JB, CC performed the experiments. JH, GG and ME planned the experiments. JB, CC performed the experiments. JH, JA, MD and EC analysed the results. JB, JH and ME wrote the paper. All authors offered a critical review of the paper.

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