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MINI REVIEW

Bacterial lipoxygenases for fatty acid oxyfunctionalization: advances and future directions

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Abstract
The shift to a biobased economy aims to reduce dependence on fossil fuels by converting biological materials, such as plant-derived and algae-derived fatty acids, into valuable compounds. The enzymatic oxyfunctionalization of fatty acids is of special interest because it enables the eco-friendly synthesis of a wide range of products with unique properties. Lipoxygenases (LOXs) are key oxyfunctionalization enzymes featuring broad substrate specificities and distinct regioselectivities. Among them, bacterial LOXs remain underexplored compared to their eukaryotic counterparts, representing an untapped resource with unique structural and functional traits. This review highlights recent advances in bacterial LOXs, emphasizing their versatile oxidative capabilities via dioxygenase and hydroperoxide isomerase activities. Key strategies, including protein engineering and oxygen level modulation, are discussed for modifying substrate specificity, regioselectivity, and preferred catalytic pathway. Multi-enzyme cascade reactions further expand the scope of LOX-derived products, unlocking new applications in biotechnological contexts. We address challenges in harnessing bacterial LOXs' biocatalytic potential and propose solutions to optimize their performance. This work enhances understanding of bacterial LOXs and highlights their potential for transformative biotechnological applications in fatty acid derivatization.

Keywords Dioxygenase · Hydroperoxide isomerase · Lipoxygenase · Oxyfunctionalization · Oleochemicals · Oxidoreductase · Oxylipins

Introduction

The growing interest in a biobased economy reflects the need to reduce reliance on fossil resources by sustainably converting renewable biomass into value-added chemicals and materials. In this context, fatty acids derived from plant and algal oils are abundant and widely available feedstocks that serve as important starting materials for the production of biobased chemicals (de Espinosa And Meier 2011). Functionalizing fatty acids through oxyfunctionalization—a process that introduces peroxyl (OOH), hydroxyl (OH), epoxyl, carbonyl (C = O), and carboxyl (COOH) groups—is crucial for enhancing their reactivity and enabling the synthesis of diverse valuable products. In addition, depending on the position of oxyfunctionalization within the fatty acid, derivative products of varying chain lengths can be obtained, which in turn affect their properties and functionalities (Revol-Cavalier et al. 2025).

One promising approach to achieve fatty acid oxyfunctionalization is through enzymatic conversion, which can be carried out by enzymes such as fatty acid hydratases (FAHs), cytochrome P450 monooxygenases (CYPs), peroxygenases (PXGs), and lipoxygenases (LOXs) (Feussner And Wasternack 1998; Konkel And Schunck 2011; Aranda et al. 2018; Bornscheuer 2018; Hagedoorn et al. 2021). Among these enzymes, lipoxygenases (LOXs) offer distinct advantages that make them highly attractive for industrial applications. Unlike CYPs, LOXs do not require costly cofactors or complex redox partner systems, significantly reducing operational costs and simplifying reaction setups, which is beneficial for large-scale processes. In addition, LOXs exhibit

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Fig. 1 Lipoxygenase-catalyzed reactions. **A** The enantio- and regioselective dioxygenation activity of LOX converts polyunsaturated fatty acids (PUFAs) containing a (1*Z*,4*Z*)-pentadiene structural unit into fatty acid hydroperoxides (FAHPs) containing a conjugated diene pattern. The dioxygenation enantioselectivity of LOX is either *R*-selective or *S*-selective. The regioselectivity shown in the figure at C5 of the pentadiene moiety is an example; alternative regioselectivity at C1 is also possible. **B** The hydroperoxide isomerase (HPI) activity of LOX converts FAHPs into epoxy alcohols or ketones

a broader substrate scope than FAHs and offer more diverse regioselectivities than FAHs, CYPs, and PXGs, resulting in the efficient production of various oxyfunctionalized fatty acids.

LOXs (EC 1.13.11.x) are non-heme iron, or sometimes manganese-dependent enzymes, that catalyze the regio- and enantioselective dioxygenation of polyunsaturated fatty acids (PUFAs) leading to the formation of fatty acid hydroperoxides (FAHPs) (Fig. 1A). FAHPs are key intermediates, serving as precursors for a range of valuable chemical compounds, including flavors and fragrances (Johannsen et al. 2020, 2021; Ribeaucourt et al. 2022), oleochemicals (Song et al. 2013; Seo et al. 2015; Yu et al. 2018; Kim And Park 2019), and lipid mediators (An et al. 2021). In addition to dioxygenation activity, a few LOXs from soybean, mammals, fungi and bacteria have been reported to have hydroperoxide isomerase (HPI) activity, which transforms FAHPs into epoxy alcohols or ketones (Fig. 1B) (Garssen et al. 1976; Yu et al. 2003; Cristea And Oliw 2006; Zheng and Brash 2010). The presence of HPI activity in LOX is of interest because hydroxy, epoxy or keto groups typically impart special characteristics to fatty acids, such as increased viscosity and reactivity, that can be used in a wide range of applications (Hou 2009). Moreover, unsaturated epoxy alcohols serve as precursors for the synthesis of biologically active molecules (Riera And Moreno 2010). The presence of HPI activity in LOXs enables further diversification of reaction products, making them uniquely capable of generating a broader range of fatty acid derivatives. Their unique combination of versatility in transforming diverse PUFA substrates and ability to generate regioselective products highlights LOXs as promising biocatalysts for biotechnological applications.

LOXs have been studied across diverse biological kingdoms, including mammals, plants, fungi, and bacteria. Most early research and reviews focused on eukaryotic LOXs, especially their biochemical characteristics (Brash 1999; Coffa et al. 2005; Andreou And Feussner 2009) and applications (Nanda And Yadav 2003; Joo and Oh 2012; Heshof et al. 2016; Hayward et al. 2017; An et al. 2021). Unlike their well-characterized eukaryotic counterparts, bacterial LOXs remained largely unexplored until the past decade, when phylogenetic analyses first proposed them as a separate subfamily with unique features (Hansen et al. 2013). Since then, increasing efforts to explore and characterize novel bacterial LOXs have

been reported (Newie et al. 2016; An et al. 2018a; Goloshchapova et al. 2018; Ellamar et al. 2019; Qi et al. 2020; Lee et al. 2022; Oh et al. 2022; Chrisnasari et al. 2024b, 2025a). These enzymes exhibit broad substrate specificity toward polyunsaturated fatty acids (PUFAs, C18–C22) and often display distinct and desirable selectivity (An et al. 2021; Chrisnasari et al. 2022).

The rapid expansion of available bacterial LOX sequences in databases (> 4700 sequences in 2022) combined with the fact that less than 0.5% have been biochemically characterized, highlights both the diversity and the significant knowledge gaps within this enzyme family. A comprehensive phylogenetic analysis of bacterial LOXs, including sequence clustering, structural prediction, and identification of residues influencing regioselectivity, has been reported in our previous review (Chrisnasari et al. 2022), in which bacterial LOXs were grouped into nine distinct phylogenetic clusters predicted to possess unique structural and functional characteristics. In addition, another review summarized the use of bacterial LOXs for the biocatalytic synthesis of lipid mediators (An et al. 2021).

Building on this foundation, this review provides a critical update on bacterial LOXs, offering deeper insights into factors influencing their catalytic reactions and innovative strategies for modulating their activity. We also explore how multi-enzyme cascade reactions expand the scope of LOX-derived products and their applications. Finally, we discuss the key challenges in harnessing the biocatalytic potential of these enzymes and propose solutions to advance their use in industrial and biotechnological contexts.

LOX-catalyzed reactions

Dioxygenase activity: factors influencing regioselectivity and substrate preference

LOX catalyzes the regioselective dioxygenation of fatty acids through five distinct steps (Fig. 2): (1) The ferric iron (Fe^{3+}) cofactor initiates the reaction by abstracting a hydrogen atom at the center of the pentadiene structure of the fatty acid substrate (Lehnert and Solomon 2003) the unpaired electron is transferred to the ferric iron and the proton is transferred to the hydroxide ligand coordinated to the iron, yielding ferrous iron (Fe^{2+}), water, and a lipid alkyl radical; (2) The unpaired electron in the lipid alkyl radical undergoes rearrangement to either the [+ 2] or the [− 2] position relative to the abstracted hydrogen; (3) A dioxygen molecule is introduced leading to the formation of a fatty acid peroxyl radical (ROO•); (4) The fatty acid peroxyl radical is then reduced by an electron from the ferrous iron (Fe^{2+}) and protonated, resulting in the formation of FAHP and the iron is re-oxidized to its ferric form (Fe^{3+}); (5) The FAHP is then released and the enzyme is ready to start the next catalytic cycle (Hamberg And Samuelsson 1967; Egmond et al. 1972; Hamberg et al. 1998; Lehnert And Solomon 2003).

The regioselectivity of LOX dioxygenation is determined by specific structural characteristics of the enzyme, such as the position and size of the oxygen migration channel (Knapp et al. 2001; Coffa and Brash 2004; Newcomer and Brash 2015; Collazo And Klinman 2016) and the depth of the substrate-binding pocket (Vogel et al. 2010). A key residue in the oxygen channel, known as the Coffa site, was found to determine the regio- and enantioselectivity of LOXs by directing the oxygen molecule through a glycine/alanine switch (Coffa and Brash 2004; Coffa et al. 2005). With respect to residues influencing the depth of the substrate-binding pocket, studies on mammalian LOXs have identified three key amino acid determinants (triad determinants): Sloane determinant (SD) (Sloane et al. 1991), Borngräber 1 determinant (BD1) (Borngräber et al. 1996), and Borngräber 2 determinant (BD2) (Borngräber et al. 1999). The role of the Coffa site and triad determinants in determining the regio- and enantioselectivity of bacterial LOXs has been summarized in our previous review (Chrisnasari et al. 2022).

The corresponding residues of triad determinants in other LOXs are typically identified through amino acid sequence alignment. However, this method is not always accurate for bacterial LOXs due to their structural heterogeneity. For instance, the crystal structures of LOX from *Pseudomonas aeruginosa* (Pa-LOX) revealed that Pa-LOX exhibits a large U-shaped substrate-binding pocket, similar to the binding pockets commonly found in LOXs from mammals, plants, and corals (Fig. 3A) (Newcomer and Brash 2015). Multiple sequence alignments identified amino acid residues corresponding to the triad determinants in Pa-LOX, i.e., E369 (BD1), M434 and F435 (SD), and I608 (BD2) (Chrisnasari et al. 2022). However, the position of BD1 was relatively distant from the bottom of the pocket, while E373 was positioned closer to it, suggesting a larger contribution of this residue in determining the depth of the binding pocket (Fig. 3A). A similar observation was made for the AlphaFold model of *Burkholderia thailandensis* LOX (Bt-LOX), which belongs to the same bacterial LOX cluster as Pa-LOX (Chrisnasari et al. 2024b).

Compared to Pa-LOX, LOX from *Cyanothece* sp. (Cya-LOX) exhibits a smaller, straight substrate-binding pocket, which is structurally very different from that of Pa-LOX and other eukaryotic LOXs (Fig. 3B). As a consequence, the residues forming the bottom of the substrate-binding pocket in Cya-LOX (Fig. 3C), i.e., E355, Y360, V365, F413, and V406, do not correspond to any of the triad determinants. A similar phenomenon is observed in the AlphaFold model

Fig. 2 Reaction mechanism of lipoxygenase (LOX). LOX catalyzes the oxygenation of fatty acids through five reaction steps: hydrogen abstraction, radical rearrangement, oxygen insertion, radical reduction-protonation, and product release. The figure is adapted from (Chrisnasari et al. 2024a) with some modification

of *Microcystic aeruginosa* LOX (Ma-LOX), which clusters with Cya-LOX within the bacterial LOX phylogeny (Chrisnasari et al. 2025a). The residues forming the bottom of the substrate-binding pocket in Cya-LOX are also positioned at the bottom of the Ma-LOX pocket, whereas the residues corresponding to the triad determinants are not located near the bottom of the binding pocket. These findings collectively indicate that identifying triad determinants in bacterial LOXs solely through amino acid sequence alignment with LOXs from other kingdoms may not be reliable. However, as demonstrated for Bt-LOX (Chrisnasari et al. 2024b), sequence alignment combined with structural modeling is useful for predicting triad determinants within the same bacterial LOX cluster.

Over the past three decades, extensive efforts have been made to modify residues at the bottom of the substrate-binding pocket to alter the dioxygenation regioselectivity of LOXs (Table 1). In mammalian LOXs, introducing larger residues typically constrains the pocket, limiting substrate entry and shifting regioselectivity toward the methyl end of the substrate (Borngräber et al. 1996). Conversely, smaller residues deepen the pocket, allowing further substrate penetration and shifting oxygenation closer to the carboxyl end (Sloane et al. 1991; Borngräber et al. 1996, 1999).

Compared to mammalian LOXs, bacterial LOXs exhibit more varied outcomes when trying to direct the regioselectivity of dioxygenation through protein engineering. For instance, introducing smaller residues at the bottom of the substrate-binding pocket in bacterial LOXs results in a loss of regioselectivity, leading to the production of hydroperoxides at random positions (An et al. 2018a). In other cases, replacement with smaller residues, as expected, can deepen the substrate-binding cavity, allowing deeper substrate penetration and shifting oxygenation closer to the carboxyl end of the fatty acid, as demonstrated for *Pseudomonas aeruginosa* LOX engineered to produce 8S-hydroperoxy and

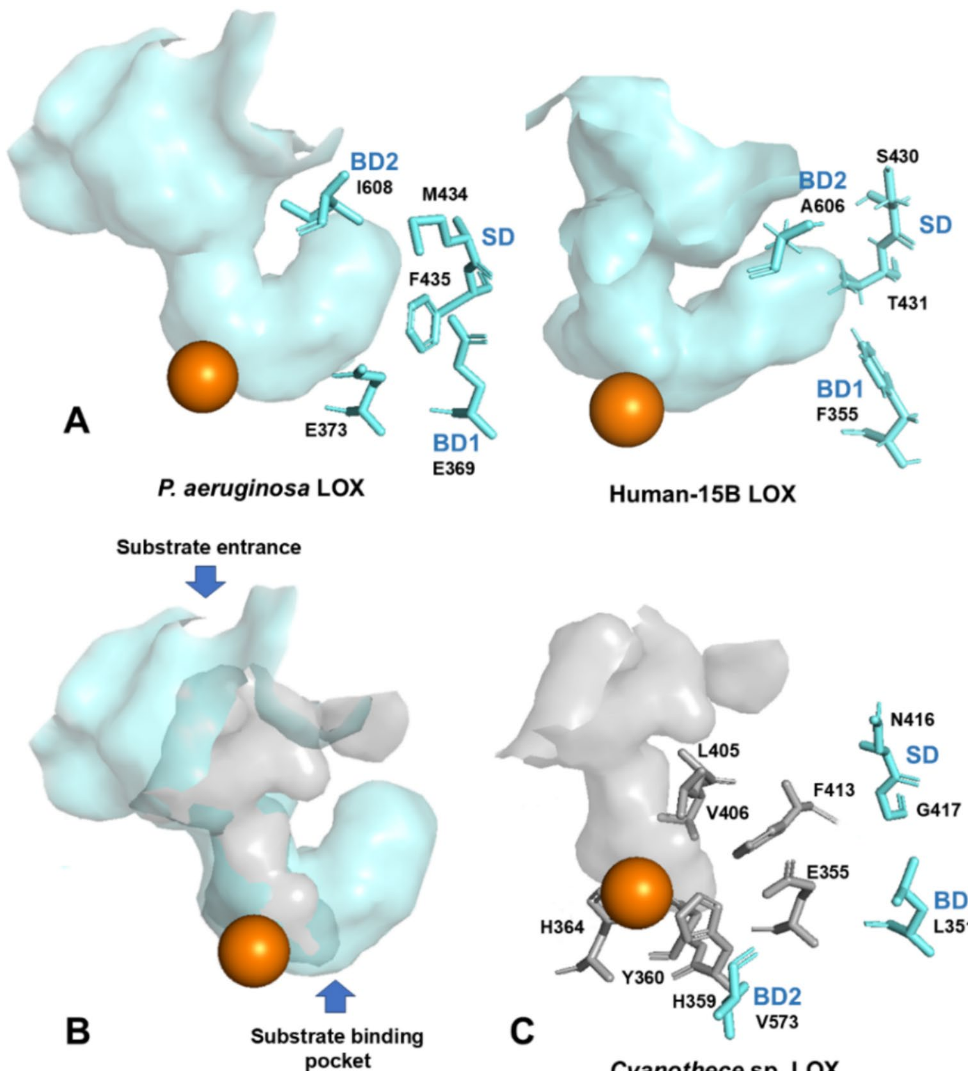


Fig. 3 Comparison of substrate-binding pocket architectures in representative bacterial and mammalian lipoxygenases. (A) Comparison of the substrate-binding pockets of *Pseudomonas aeruginosa* LOX (Pa-LOX, PDB 5IR5) and human 15B-LOX (PDB 4NRE). Both enzymes exhibit a relatively large U-shaped substrate-binding pocket typical of many lipoxygenases. Residues forming the bottom triad determinants (BD1, SD, BD2), which are associated with control of regioselectivity, are indicated. Despite the similar overall pocket architecture, a different relative position of BD1 can be observed. (B) Superimposition of the substrate-binding pockets of Pa-LOX and *Cyanothece* sp. LOX (Cya-LOX, PDB 5EK8). Pa-LOX displays a larger U-shaped channel (cyan), whereas Cya-LOX exhibits a narrower and more linear substrate channel (grey), illustrating structural diversity among bacterial LOXs that may influence substrate positioning and oxygenation patterns. (C) Residues located at the bottom of the Cya-LOX substrate-binding pocket, including the corresponding triad determinant positions (BD1, SD, BD2). The iron cofactor is shown as an orange sphere

11*S*-hydroperoxy eicosatetraenoic acid (Seo et al. 2025). However, such modifications do not universally alter regioselectivity; in some instances, mutations have little or no effect on regioselectivity or may even lead to enzyme inactivation (Banthiya et al. 2016; Goloshchapova et al. 2018; Chrisnasari et al. 2024b). An unexpected shift in regioselectivity toward the methyl end has been observed for the L445A variant of Bt-LOX. Especially with ω−3 PUFAs, this SD variant demonstrated a shift in the preferred dioxygenation position from the ω−5 to the ω−2 carbon, a regioselectivity that has not been reported for any other LOX before (Chrisnasari et al. 2024b). Structural modeling and docking experiments suggested that the L445A substitution caused a smaller and reshaped substrate-binding pocket, promoting a shallower penetration for ω−3 PUFAs. With several ω−6 PUFAs, the L445A variant of Bt-LOX facilitated dioxygenation closer to the carboxylic acid terminus, indicating a shift in substrate orientation (Chrisnasari et al. 2024b), consistent with observations reported for *Myxococcus xanthus* LOX (An et al. 2018a).

Given the complexity of substrate-binding pocket dynamics, the effects of bottom-pocket residue substitutions cannot be universally generalized across all LOXs. Structural changes introduced by these substitutions can alter the pocket's shape, size, and residue positioning, resulting in diverse regioselectivity outcomes. Therefore, detailed product profiling, structural analysis, and computational modeling are crucial for understanding and predicting the effects of such modifications in individual LOXs.

Besides affecting regioselectivity, the shape and size of the substrate-binding pocket likely explain the distinct substrate specificities observed among bacterial LOXs. Pa-LOX and Bt-LOX, which have spacious U-shaped substrate-binding pockets, prefer ω−6 and longer PUFAs (Banthiya et al. 2016; Chrisnasari et al. 2024b). In contrast, Cya-LOX, with its smaller and straighter pocket, is active toward linoleic

Table 1 Effects of bottom pocket residue substitutions on LOX regioselectivity

Enzyme	Enzyme variant*	Regioselectivity (%) **						Ref
		15-	**12-**	**11-**	**9-**	**8-**	**5-**	
Mammalian LOXs								
Human 15-LOX	Wild-type	90	10	-	-	-	-	(Sloane et al. 1991)
	M 418 V (SD)	60	40	-	-	-	-	
	M418V/**I**417A/Q416**K** (SD)	5	95	-	-	-	-	
Rabbit reticulocyte 15-LOX	Wild-type	97	3	-	-	-	-	(Borngräber et al. 1996)
	F353L (BD1)	28	72	-	-	-	-	
	F353L/I593**Y** (BD1/BD2)	21	79	-	-	-	-	(Borngräber et al. 1999)
Porcine leukocyte 12-LOX	Wild-type	3	97	-	-	-	-	(Borngräber et al. 1996)
	L353**F** (BD1)	94	6	-	-	-	-	
Human 12/15-LOX	Wild-type	86	14	-	-	-	-	(Vogel et al. 2010)
	F353L (BD1)	12	88	-	-	-	-	
	I 418 A (SD)	6	94	-	-	-	-	
	M 419 V (SD)	42	58	-	-	-	-	
	I 593 A (BD2)	65	35	-	-	-	-	
	F353L/**I** 418 F (BD1/SD)	64	36	-	-	-	-	
	F35L/**I**418F/**I** 593 A (BD1/SD/BD2)	70	30	-	-	-	-	
P. pygmaeus 12/15-LOX	Wild-type	86	14	-	-	-	-	(Vogel et al. 2010)
	F 353 V (BD1)	6	94	-	-	-	-	
	I 418 A (SD)	18	82	-	-	-	-	
	M 419 V (SD)	60	40	-	-	-	-	
	I 593 A (BD2)	60	40	-	-	-	-	
Bacterial LOXs								
Myxococcus xanthus LOX	Wild-type	10	90	-	-	-	-	(An et al. 2018a)
	T397**Y** (BD1)	94	6	-	-	-	-	
	T 397 A (BD1)	28	11	-	-	26	35	
	A461**I** (SD)	33	18	-	-	13	36	
	A461**F** (SD)	41	40	-	-	3	16	
	I664**Y** (BD2)	35	65	-	-	-	-	
Myxococcus fulvus LOX	Wild-type	5	95	-		-		(Goloshchapova et al. 2018)
	G359**F** (BD1)	-	-	-	-	-	-	
	F424I/**I**425M (SD)	5	95	-		-		
Pseudomonas aeruginosa LOX	Wild-type	100	-	-	-	-	-	(Banthiya et al. 2016)
	E 369 A (BD1)	100	-	-	-	-	-	
	M 434 V (SD)	100	-	-	-	-	-	
	F435L (SD)	100	-	-	-	-	-	
	L612V	100	-	-	-	-	-	
Pseudomonas aeruginosa LOX	Wild-type	97	-	-	-	-	3	(Seo et al. 2025)
	M434G (SD)	6	-	61	-	20	13	
	Y609G	2	-	-	-	98	-	
	Y609A	-	-	-	-	100	-	
	Y609V	6	-	-	-	88	6	
Burkholderia thailandensis LOX	Wild-type	98	2		-	-	-	(Chrisnasari et al. 2024b)
	I 618 A (BD1)	-	-		-	-	-	
	L 445 A (SD)	46	3		2	6	43	
	F 446 V (SD)	96	4		-	-	-	

*Larger residue is indicated in bold. Corresponding residues to the triad determinants are indicated in parentheses

**Reported regioselectivity when using arachidonic acid (AA, C20:4 ω−6) as substrate. Regioselectivity (%): percentage of product formed at a specific oxygenation position relative to the sum of all regioisomers formed

acid (LA, C18:2 ω−6), α-linolenic acid (ALA, C18:3 ω−3), arachidonic acid (AA, C20:4 ω−6), and eicosapentaenoic acid (EPA, C20:5 ω−3), although its precise substrate preference remains unreported (Newie et al. 2016). Ma-LOX has a similarly small pocket and shows a preference for LA and ALA over longer PUFAs (Chrisnasari et al. 2025a). These findings highlight the critical role of pocket shape and size in determining the substrate specificity of LOX.

Bacterial LOXs generally exhibit distinct preferences for specific PUFA substrates. For instance, LOXs from *Myxococcus fulvus* and *Pseudomonas aeruginosa* PAO1 preferentially act on longer-chain PUFAs (Banthiya et al. 2016; Goloshchapova et al. 2018). In contrast, LOX from *Microcystic aeruginosa* demonstrates a strong preference for shorter-chain PUFAs (Chrisnasari et al. 2025a). In addition, LOXs from *Burkholderia thailandensis*, *Pseudomonas* sp. 42A2, *Calothrix* sp. HK-06, *Sphingopyxis macrogoltabida*, and *Myxococcus xanthus* demonstrate high activity towards LA, but reduced activity towards ALA (Busquets et al. 2004; An et al. 2015, 2018a; Qi et al. 2020; Kim et al. 2022; Chrisnasari et al. 2024b).

Given the inherent substrate preferences of LOXs, the ability to engineer these enzymes to modify their substrate specificity has great potential for broadening their applications. A striking example involves the L445A substitution in Bt-LOX. As explained above, this substitution caused a positional shift in nearby residues, reducing the size of the substrate-binding pocket and changing its shape. These changes altered the enzyme's substrate penetration depth and resulted in a shift in substrate preference from ω−6 PUFAs to ω−3 PUFAs (Chrisnasari et al. 2024b).

Double dioxygenase activity and its possible mechanisms

In addition to catalyzing single dioxygenation reactions, some LOXs also demonstrate a double dioxygenation reaction. Double dioxygenation reactions are of particular interest due to the potential anti-inflammatory effects of the derived dihydroxy fatty acids (Basil And Levy 2015; Ishihara et al. 2019). Recently, several bacterial LOXs have been reported to demonstrate a pronounced double dioxygenation activity (Kim et al. 2021, 2022; Lee et al. 2022; Oh et al. 2022). Despite these intriguing findings, the precise mechanisms underlying double dioxygenation in LOXs remain poorly understood. The initial explanation for double dioxygenation reactions focused on variations in substrate orientation within the enzyme's binding pocket, as reported for soybean LOX-1, which catalyzes the formation of 5,15-dihydroperoxy fatty acids from arachidonic acid (AA, C20:4 ω−6) (Van Os et al. 1981). In this scenario, the initially formed 15-hydroperoxy fatty acid re-enters the enzyme in a head-first orientation and undergoes a second oxygenation at the C5 position to generate the double dioxygenation product (Fig. 4A). While this scenario provides an interesting perspective, it fails to account for the distinct dioxygenation patterns observed in other LOXs, including the two bacterial LOXs discussed below.

An alternative explanation suggests that variations in substrate penetration depth play a key role in double dioxygenation, as reported for *Sphingopyxis macrogoltabida* LOX (Sm-LOX), which generates 9S,15S-dihydroperoxy and 11*S*,17*S*-dihydroperoxy fatty acids from C20 and C22 PUFAs, respectively (Kim et al. 2022). In this case, when using AA as a substrate, the enzyme first oxygenates at the C9 position to produce a 9-hydroperoxy fatty acid. This product then re-enters the binding pocket, where a second

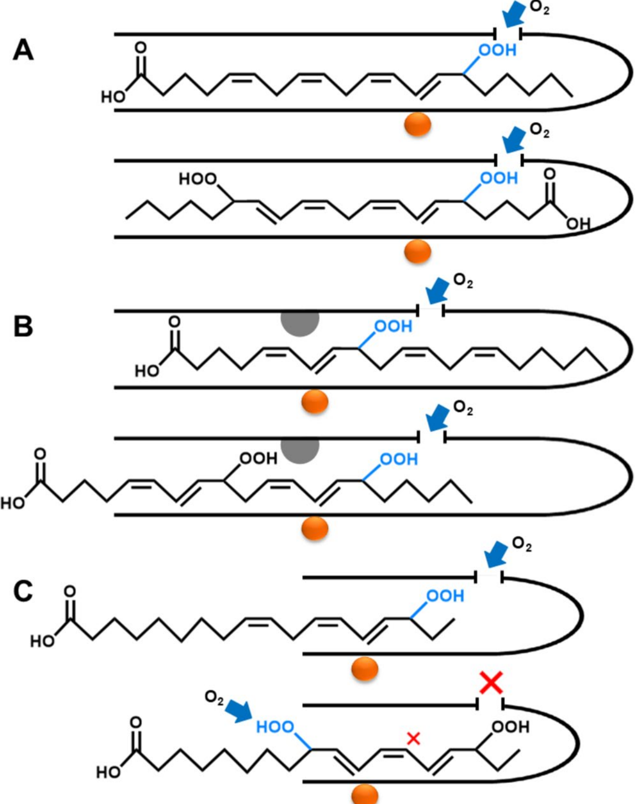


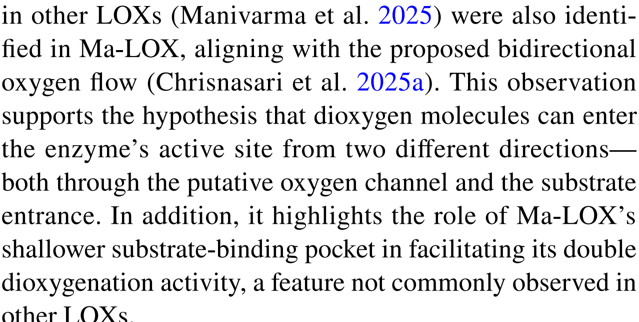
Fig. 4 Proposed mechanisms for double dioxygenation activity in LOXs. **A** Initially, double dioxygenation was ascribed to variations in substrate orientation within the binding pocket (carboxyl end-first or methyl end-first). **B** Another potential mechanism involves differences in substrate penetration depth, caused by a blocking residue (shown in grey semicircle). **C** Recently, a novel mechanism for double dioxygenation in LOXs has been proposed. A shallow substrate-binding pocket may enable oxygen insertion via two distinct pathways: through the substrate entrance and a putative oxygen channel. The direction of oxygen insertion is indicated by blue arrows, and the iron cofactor is shown as an orange sphere. Newly added hydroperoxide groups are highlighted in blue

oxygenation occurs at the C15 position. The hydroperoxide group at C9 hinders further substrate penetration, in part due to the presence of a blocking residue in the pocket, preventing oxygenation at other positions (Kim et al. 2022) (Fig. 4B).

Another mechanism for the LOX-mediated double dioxygenation reaction has been proposed for Ma-LOX (Chrisnasari et al. 2025a). The AlphaFold model of Ma-LOX revealed a shallow substrate-binding pocket, which suggests that oxygen insertion may occur via two distinct pathways, through the substrate entrance and a putative oxygen channel. This mechanism leads to the formation of 9,16-dihydroperoxy fatty acids from α-linolenic acid (ALA; C18:3 ω−3). Upon the first oxygenation at the C16 position producing a 16-hydroperoxy fatty acid, the hydroperoxide group at this position prevents further oxygen insertion through the oxygen channel. Consequently, oxygenation at the C13 position is blocked, and instead, oxygen insertion occurs at the C9 position through the substrate entrance (Fig. 4C). The same principle applies when the first dioxygenation occurs at the C9 position; the hydroperoxide group at this position hinders the insertion of oxygen from the substrate entrance, thus the dioxygenation at C12 is not possible. However, oxygen insertion from the putative oxygen channel to the C16 position is still possible. This deviating mechanism underscores the complexity of double dioxygenation activity in LOX catalysis.

The oxygen diffusion pathway in LOX catalysis has been previously studied through molecular dynamics simulations. In soybean LOX-1, eight possible channels for oxygen diffusion have been identified. However, computational and mutagenesis studies strongly support only one dominant channel that directs molecular oxygen to a specific region of the active site, ensuring the regio- and enantio-specificity of the product (Collazo And Klinman 2016). Similar to soybean LOX, studies on mammalian LOXs have identified four oxygen channels. However, only one channel—localized opposite to the nonheme iron—exhibits high oxygen affinity at the active site (Saam et al. 2007). The critical role of an oxygen channel opposite to the nonheme iron in determining LOX catalytic specificity has also been proposed in some reviews (Kuhn et al. 2005; Newcomer and Brash 2015).

A recent study reported the presence of two primary oxygen channels that direct oxygen to the substrate-binding pocket, based on conserved motifs and helical structures found across the LOX family, including plants, mammals, and bacteria (Manivarma et al. 2025). However, these two primary oxygen channels predominantly lead to the formation of a single oxidized product, indicating that only one channel is primarily responsible for LOX catalysis, while the other is located relatively far from the non-heme iron. Interestingly, the two conserved motifs and structures observed in other LOXs (Manivarma et al. 2025) were also identified in Ma-LOX, aligning with the proposed bidirectional oxygen flow (Chrisnasari et al. 2025a). This observation supports the hypothesis that dioxygen molecules can enter the enzyme's active site from two different directions—both through the putative oxygen channel and the substrate entrance. In addition, it highlights the role of Ma-LOX's shallower substrate-binding pocket in facilitating its double dioxygenation activity, a feature not commonly observed in other LOXs.



The variability of proposed double-dioxygenation mechanisms across different LOXs highlights the complexity of this catalytic activity. Different enzymes appear to adopt a distinct strategy to achieve double dioxygenation, suggesting that no single model can be universally applied. Therefore, unraveling the mechanisms driving double dioxygenation requires a comprehensive approach, combining detailed product profiling, structural analysis, and computational modeling to predict enzyme-substrate interactions. Only through this integrated approach can we begin to fully understand the unique catalytic capabilities of individual LOXs and their potential applications in biocatalysis.

Factors affecting hydroperoxide isomerase activity (HPI)

In addition to their primary dioxygenase activity, a few LOXs from soybean, mammals, fungi, and bacteria have been reported to have hydroperoxide isomerase (HPI) activity (Garssen et al. 1976; Yu et al. 2003; Cristea And Oliw 2006; Zheng and Brash 2010; Chrisnasari et al. 2025a, 2025b). These two reactions are catalyzed by LOX at different oxidation states of the non-heme iron: dioxygenase activity requires the ferric (Fe^{3+}) state, while HPI activity requires the ferrous (Fe^{2+}) state. Newly isolated enzymes are typically in the ferrous state, which is inactive toward PUFAs. They require a priming reaction with FAHPs to convert the iron to its ferric state (Schilstra et al. 1992, 1993), thereby, activating the enzyme to enter the dioxygenase catalytic cycle (Schilstra et al. 1994) (Fig. 5). An alkoxyl radical or epoxy-allylic radical is postulated as an intermediate in the LOX activation process (Yu et al. 2003; Zheng and Brash 2010). The alkoxyl radical or epoxy-allylic radical subsequently dissociates through an oxygen-dependent dissociation process, leading to the formation of epoxy-allylic hydroperoxides, which are then further transformed into epoxy-allylic ketones (Zheng and Brash 2010). LOX activation is a single-turnover event, meaning that once the enzyme is oxidized to its ferric form, it will remain in this form and no longer react with FAHPs (de Groot et al. 1975). An exception applies to LOXs that exhibit HPI activity, as the ferrous form of these enzymes can convert FAHPs into

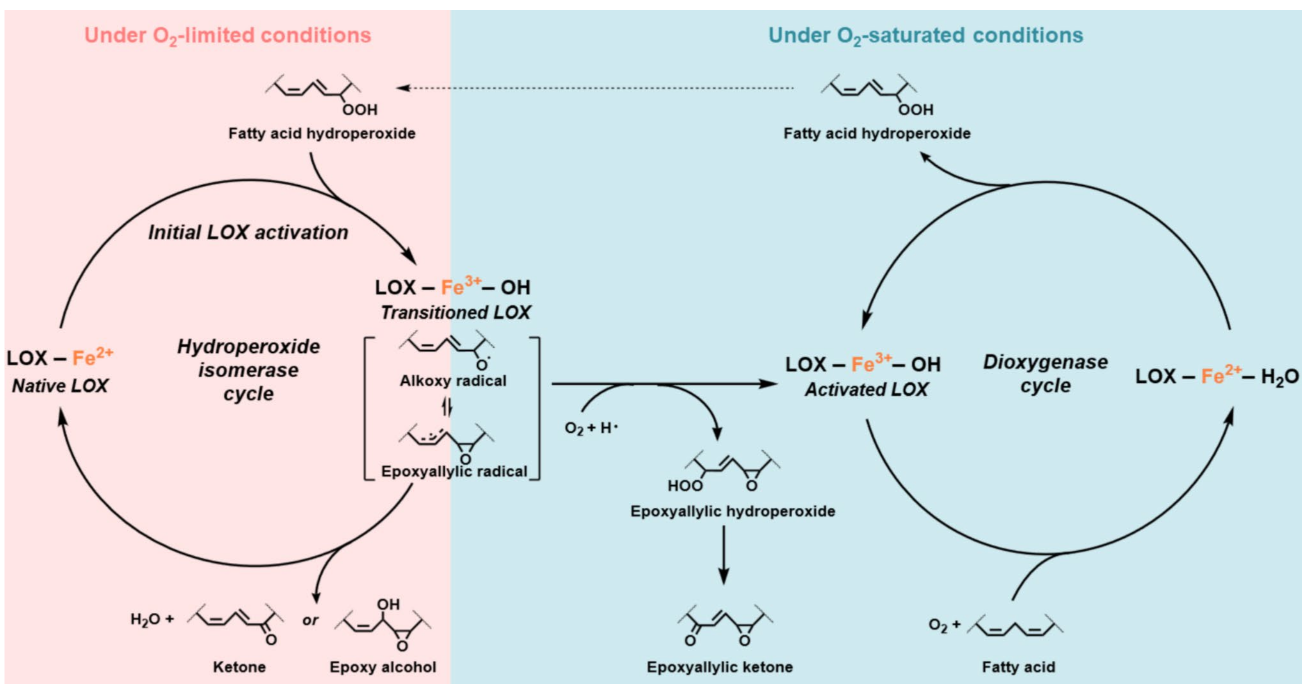


Fig. 5 Modulating dioxygenase and hydroperoxide isomerase (HPI) activity of LOX. Activation of the non-heme iron cofactor of LOX by FAHP (typically generated through auto-oxidation) leads to the formation of an alkoxy-allylic or epoxy-allylic radical intermediate. In the presence of oxygen, these radical intermediates dissociate via an oxygen-dependent pathway to form an epoxy-allylic hydroperoxide, which is subsequently transformed into an epoxy-allylic ketone. The free ferric enzyme then catalyzes a dioxygenase reaction, converting PUFA into FAHP. LOX activation is a single-turnover event, meaning that once the enzyme is oxidized to its ferric form, it will remain in this form and no longer react with FAHPs. Under O_2-saturated conditions, both the oxygen-dependent dissociation of the radical intermediates and the dioxygenase reaction become more pronounced. Conversely, under O_2-limited conditions, the radical intermediate can be transformed into an epoxy alcohol or ketone by HPI activity, with the iron in LOX being regenerated to its ferrous state upon product release. When continuous regeneration of the ferrous enzyme occurs due to HPI activity, the FAHP produced by dioxygenase activity can enter the HPI cycle and be further transformed into either an epoxy alcohol or ketone (indicated by a dashed arrow). This figure is adopted from Chrisnasari et al. (2025b)

epoxy alcohols or ketones, reverting to their ferrous state after product release (Yu et al. 2003; Zheng and Brash 2010).

HPI activity in those LOXs typically occurs only under specific conditions. For instance, soybean LOX-1 exhibits HPI activity in the presence of excess FAHP and under anaerobic conditions (Garssen et al. 1976). HPI activity in the manganese LOX from *Gaeumannomyces avenae* is induced by the G316A mutation, which appears to reduce the size of the oxygen channel, limiting oxygen access and promoting HPI activity (Cristea And Oliw 2006). Human epidermal LOX3 (eLOX3), despite being an isoform of human 15-LOX2 with its high dioxygenase activity (Kobe et al. 2014), is atypical as it either entirely lacks dioxygenase activity (Yu et al. 2003) or requires a prolonged lag phase to initiate it (Zheng and Brash 2010). The absence of an oxygen channel in this enzyme may explain its high HPI activity and lack of dioxygenase activity (Fig. S1). Bacterial LOXs from *Burkholderia thailandensis* and *Microcystic aeruginosa* exhibit strong HPI activity at high enzyme concentrations (Chrisnasari et al. 2025a, 2025b). From this, it was concluded that elevated enzyme concentrations increase oxygen consumption, thereby creating oxygen-limited conditions that favor HPI activity.

The dual functionality of LOXs, which exhibit both dioxygenation and HPI activity, expands the catalytic repertoire of these enzymes but also poses challenges in product purification. The products of dioxygenase activity (FAHPs) and the products of HPI activity (epoxy alcohols or ketones) have different potential applications which will be discussed in the next section. Harnessing the full potential of LOXs requires a deeper understanding of how to control their activity, steering the enzyme toward either the dioxygenase or HPI pathway, depending on the desired outcome. A study on Bt-LOX revealed that by controlling oxygen concentration in the reaction environment, HPI and dioxygenase activity can be modulated (Chrisnasari et al. 2025b) (Fig. 5). Under O_2-saturated conditions, HPI activity was suppressed, while dioxygenase activity was enhanced (Faillace et al. 2025; Chrisnasari et al. 2025b). Conversely, under O_2-limited conditions, dioxygenase activity persisted, and the resulting fatty acid hydroperoxides were efficiently converted into epoxy alcohols and

ketones. It appears that the low residual oxygen levels initially present support the conversion of PUFAs into their corresponding FAHPs, which are subsequently transformed into epoxy alcohols and ketones due to pronounced HPI activity in an oxygen-limited environment. These findings highlight an effective way to modulate LOX activity and direct the enzyme toward a specific catalytic pathway. Modulating LOX activity represents a major advancement in PUFA derivatization, broadening both the product scope and the applicability of LOXs.

Potential applications of LOXs

LOX in the production of flavors and fragrances

Flavors and fragrances (F&F) are important components in consumer goods such as food, cosmetics, and household products. While chemical synthesis dominates F&F production due to its efficiency, it comes with drawbacks such as high energy consumption, toxic catalysts, and reliance on fossil-based materials (Johannsen et al. 2020, 2021; Ribeaucourt et al. 2022). This has led to increasing demand for natural alternatives in F&F production. Natural extraction methods, such as distillation, often yield extremely low amounts, driving the demand for sustainable biotechnological alternatives. Biotransformation using enzymes is an emerging and highly desirable approach to meet this demand.

Among F and F compounds, fatty aldehydes and alcohols constitute a prominent class of molecules widely used in food and perfume industries, most notably those in the C3–C12 range (Johannsen et al. 2020, 2021; Ribeaucourt et al. 2022). Fatty aldehydes of different chain lengths exhibit distinct scents (Ribeaucourt et al. 2022). Various of these aldehydes are potentially accessible from fatty acids, but their synthesis requires regioselective biocatalysts capable of generating a specific aldehyde. The diverse regioselectivity and broad substrate preferences observed in bacterial LOXs (Chrisnasari et al. 2022) enable the enzymatic production of fatty aldehydes with varying chain lengths (Table 2).

Table 2 Potential application of bacterial LOXs for fatty aldehydes production

Bacterial LOX source	Ref.	Regio-selectivity	PUFA Substrate*	Fatty aldehydes	Structure	Scent
L445A variant *B. thailandensis*	(Chrisnasari et al. 2024b)	ω−2 LOX	ALA, EPA	Propanal, Propionaldehyde		floral and fruity
P. aeruginosa	(Banthiya et al. 2016; Deschamps et al. 2016)	ω−5 LOX	LA, ALA, GLA, AA	Hexanal, Caproaldehyde		Green, grassy fruity
B. thailandensis	(An et al. 2015; Sim et al. 2015; Chrisnasari et al. 2024b)					
M. xanthus	(An et al. 2018a)					
Rivularia sp.	(Qi et al. 2020)					
Calothrix sp.						
Tolypothrix bouteillei						
Microcystis aeruginosa	(Chrisnasari et al. 2025a)					
Cyanothece sp.	(Newie et al. 2016)	ω−6 LOX	ALA			
A. marina	(Gao et al. 2010)					
M. xanthus	(An et al. 2018a)	ω−8 LOX	AA, EPA, DHA	Nonanal, Pelargonal-dehyde		Soapy, citrus-like, rose-like, floral
M. fulvus	(Goloshchapova et al. 2018)					
Nostoc sp.	(Andreou et al. 2008)	ω−9*R* LOX	LA			
Cyanothece sp.	(Newie et al. 2016)					
A. marina	(Gao et al. 2010)					
S. macrogoltabida	(Kim et al. 2022)	ω−9*S* LOX	LA, ALA			
S. macrogoltabida	(Kim et al. 2022)	ω−11 LOX	AA, EPA, DPA, DHA	Dodecanal, Lauraldehyde		Waxy, fatty, soapy, reminiscent of violets

*PUFA substrates: linoleic acid (LA, C18:2 ω−6), α-linolenic acid (ALA, C18:3 ω−3), γ-linolenic acid (GLA, C18:3 ω−6), arachidonic acid (AA, C20:4 ω−6), eicosapentaenoic acid (EPA, C20:5 ω−3), docosapentaenoic acid (DPA, C22:5 ω−3), docosahexaenoic acid (DHA, C22:6 ω−3)

FAHPs generated by LOXs can be enzymatically cleaved into aldehydes and oxo-fatty acids by hydroperoxide lyases (Brühlmann et al. 2013; Brühlmann And Bosijokovic 2016) (Fig. 6). The aldehydes may be further converted into the corresponding alcohols by alcohol dehydrogenases (ADHs), which can provide desirable sensory properties or improved stability for certain applications. Such enzymatic strategies may be particularly attractive for food applications, where high selectivity and mild reaction conditions are desirable. Alternatively, hydrogenation, i.e., chemical reduction using metal catalysts, enables both saturation of remaining carbon-carbon double bonds and reduction of aldehydes to alcohols, thereby offering an efficient route for converting unsaturated aldehydes into saturated alcohols (Milone et al. 2007; Ide et al. 2012), which is especially relevant for non-food applications. The remaining oxo-fatty acids can be further transformed into oleochemicals such as ω-hydroxy fatty acids, α,ω-dicarboxylic acids, and ω-amino carboxylic acids, as discussed in the next section. By leveraging their diverse regioselectivities and broad substrate preferences, bacterial LOXs emerge as highly promising biocatalysts for sustainable F&F production.

LOX in the production of oleochemicals

The FAHPs generated by LOXs could potentially serve as key intermediates in producing a wide range of oleochemicals, including medium-chain-length to long-chain-length ω-hydroxy fatty acids, ω-amino-carboxylic acids, and α,ω-dicarboxylic acids (Yadav and Srinivas 1997; Köckritz And Martin 2011; Song et al. 2013; Seo et al. 2015; Yu et al. 2018; Kim And Park 2019). These oleochemicals are essential for manufacturing diverse chemical products and intermediates, such as nylons and other polyamides, polyesters, resins, hot-melt adhesives, powder coatings, corrosion inhibitors, lubricants, plasticizers, and greases. Importantly, the chain length of these oleochemicals, which affects their properties and functionalities, depends on the position of the hydroperoxide group on the fatty acid. By selecting specific regioselective LOXs and fatty acid substrates, products of varying lengths can be achieved.

FAHPs generated by LOXs can be cleaved into their oxo-fatty acid derivatives using hydroperoxide lyases, which can then be further converted biocatalytically into ω-hydroxy fatty acid; α,ω-dicarboxylic acid, and ω-amino carboxylic

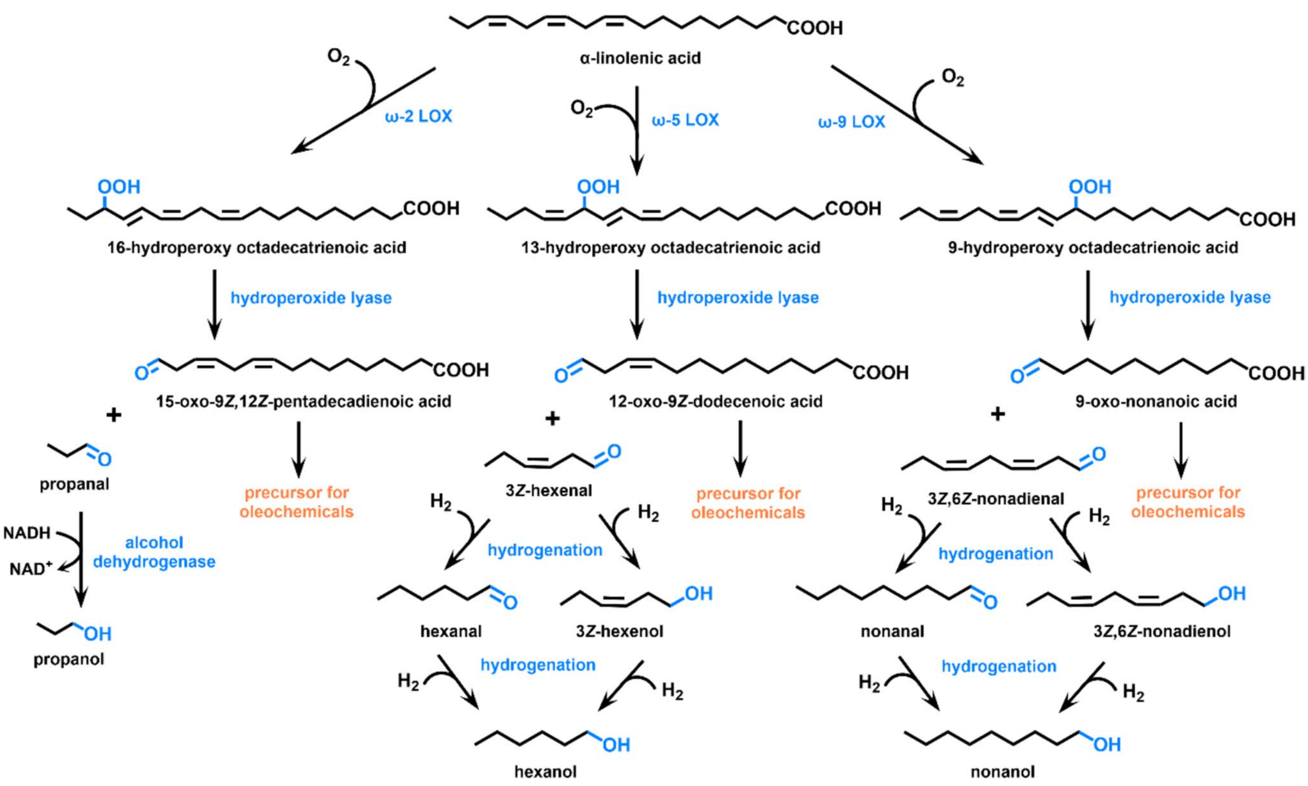


Fig. 6 Schematic multistep enzymatic synthesis of PUFA-derived aldehydes and alcohols involving distinct regioselective lipoxygenases, hydroperoxide lyases, and alcohol dehydrogenases. Hydrogenation efficiently converts unsaturated aldehydes into saturated alcohols by facilitating both the saturation of unsaturated bonds and the reduction of aldehydes to alcohols. Enzymes and newly introduced functional groups are highlighted in blue. Some of these reaction cascades are hypothetical in the sense that the regioselectivity of enzymes catalyzing the follow-up steps, i.e., hydroperoxide lyases, may have to be identified or developed

acid using alcohol dehydrogenase, aldehyde dehydrogenase, and ω-amino transferase, respectively (Song et al. 2013; Seo et al. 2015) (Fig. 7). To saturate the remaining unsaturated bonds in the resulting products, chemical hydrogenation can be performed (Žula et al. 2022; Quaranta et al. 2022; Spiekermann And Seidensticker 2024).

A significant innovation has been achieved through the L445A variant of Bt-LOX due to its unique regioselectivity at the ω − 2 position, enabling the production of ω − 2 FAHPs from ω−3 PUFAs (Chrisnasari et al. 2024b). This novel regioselectivity expands the range of fatty acid transformations by facilitating the synthesis of longer-chain oxo-fatty acids (Fig. 6). These oxo-fatty acids can subsequently be converted into ω-hydroxycarboxylic acids, α,ω-dicarboxylic acids, and ω-amino carboxylic acid derivatives (Björkhem 1972; Grechkin and Hamberg 2004; Grechkin et al. 2006), which were previously unattainable with natural LOX regioselectivities. Such extended-chain compounds hold considerable promise as precursors for biobased polymers and specialty chemicals. By demonstrating both natural and novel regioselectivities, bacterial LOXs serve as promising biocatalysts for sustainable oleochemical production, thus developing eco-friendly alternatives for the chemical industry.

LOX for the production of PUFAs-derived oxylipins

PUFA-derived oxylipins are structurally diverse and biologically active oxygenated molecules found across various organisms, including animals, plants, and microorganisms. These compounds play a critical role as signaling molecules in physiological processes such as homeostasis, cell communication, and responses to inflammation and infections (Blée 2002; Serhan 2014; Zhang et al. 2025; De Bartolo et al. 2025). In mammals, oxylipins are often referred to as lipid mediators, and include leukotrienes, lipoxins, hepoxilins, trioxilins, resolvins, protectins, and maresins. These LOX-derived oxylipins are composed of dihydroxy or trihydroxy fatty acids, and fatty acid epoxy alcohols (Fig. 8) (An et al. 2021; Lee et al. 2025). The specific functionality of these oxylipins depends on the chain-length and the degree of unsaturation of the PUFA substrate as well as the type, position, and chirality of the attached functional group (An et al. 2021; Revol-Cavalier et al. 2025; Lee et al. 2025). Research suggests that most biologically active oxylipins exist in different chiral forms and their bioactivity appears to be influenced by their chirality (Oh et al. 2011; Isobe et al.

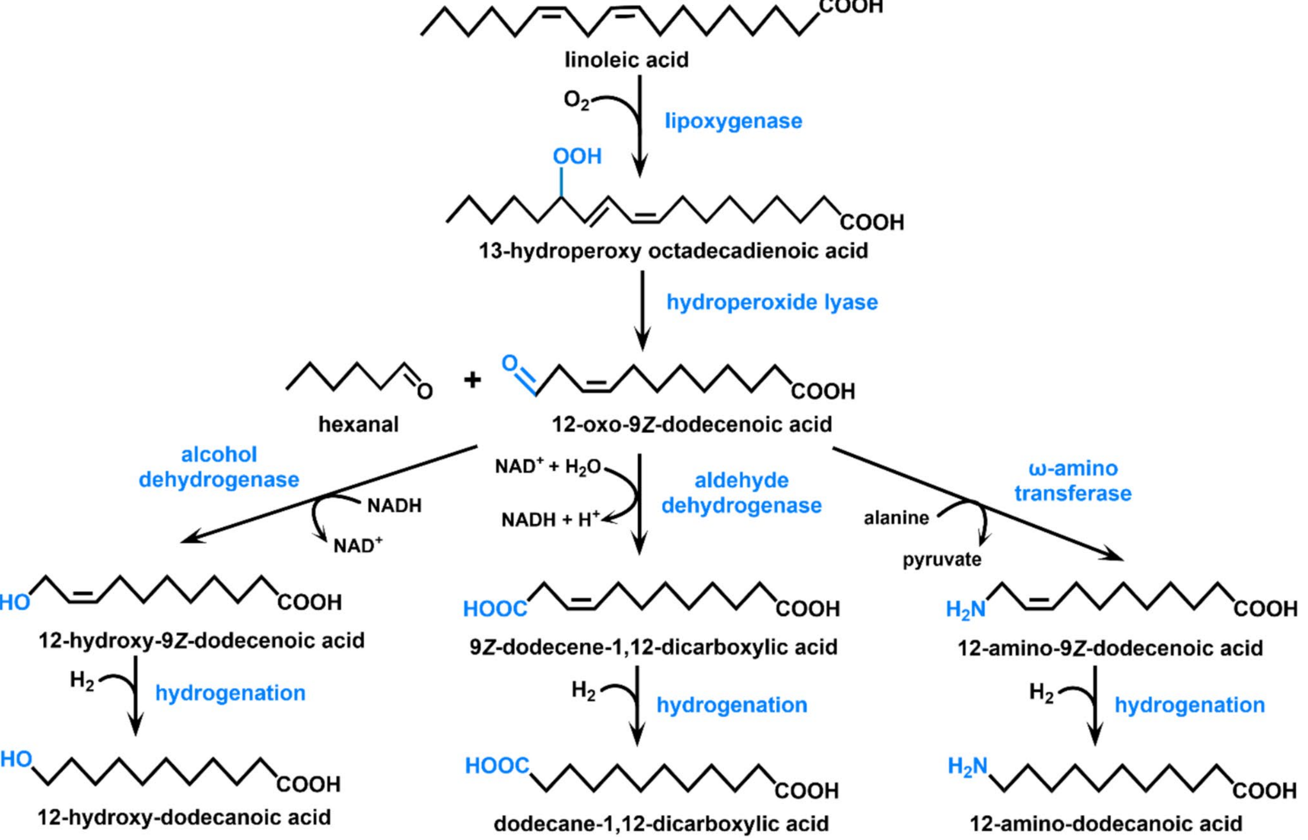


Fig. 7 Schematic representation of the multistep enzymatic synthesis of PUFA-derived ω-hydroxy fatty acids, α,ω-dicarboxylic acids, and ω-amino-carboxylic acids. The final hydrogenation step is typically carried out using chemical methods

Fig. 8 Typical chemical structures of oxylipins derived from lipoxygenase (LOX), including leukotriene (LT), lipoxin (LX), hepoxilin (Hx), trioxilin (TrX), resolvin (Rev), protectin D (PD), and maresin (MaR)

2013; Blum et al. 2019; Ambaw et al. 2020). As a result, there is significant interest in producing enantioselective oxylipins through enzymatic catalysis.

Bacterial LOXs are particularly promising for synthesizing diverse oxylipins due to their distinct substrate preferences, regio- and enantioselectivities (An et al. 2018b; Chrisnasari et al. 2022). Harnessing both the dioxygenase and HPI activities of LOX enables the generation of a broad spectrum of bioactive oxylipins (Fig. 9), especially if subsequent enzymatic or chemical derivatization processes can preserve the chirality of the compounds.

The regioselective dioxygenation of PUFAs by LOX produces specific FAHPs, which can undergo a second dioxygenation step to form dihydroperoxy fatty acids. These intermediates are then reduced to dihydroxy fatty acids either enzymatically or through chemical reduction. Some bacterial LOXs have been shown to exhibit double dioxygenation activity (Kim et al. 2021, 2022; Lee et al. 2022; Oh et al. 2022), which represents a promising green route for synthesizing dihydroxy fatty acids. The growing interest in these compounds, particularly due to their potential anti-inflammatory properties (Basil And Levy 2015; Ishihara et al. 2019), highlights the significance of bacterial LOXs as powerful tools in oxylipin biosynthesis.

An alternative route for oxylipin production involves the epoxidation of FAHPs via the HPI activity of LOX, resulting in fatty acid epoxy alcohols. Depending on the positions of the epoxy and hydroxy groups, these epoxy

Fig. 9 Schematic multistep enzymatic synthesis of PUFA-derived oxylipins involving lipoxygenases and epoxide hydrolase. Reduction of fatty acid hydroperoxide to fatty acid hydroxide (indicated by dashed arrow) can be done enzymatically or chemically

alcohols can function as biologically active molecules known as hepoxilins (An et al. 2018b). Further hydrolysis of the epoxide group by epoxide hydrolases produces trihydroxy fatty acids, another class of biologically significant molecules called trioxilins (Riera And Moreno 2010; An et al. 2018b). Both hepoxilins and trioxilins have garnered significant attention in recent years due to their anti-inflammatory, anti-infective, antibacterial, antiviral, anti-apoptotic, neuroprotective, and tissue-healing properties (An et al. 2018b). LOXs that exhibit dual functionality of dioxygenase and HPI activities, e.g., LOXs from soybean, *Gaeumannomyces avenae*, *Burkholderia thailandensis*, and *Microcystis aeruginosa* (Garssen et al. 1976; Cristea And Oliw 2006; Chrisnasari et al. 2025a, 2025b), facilitate the efficient synthesis of oxylipins by enabling the direct conversion of PUFAs into epoxy alcohols. The dual functionality of these LOXs underscores their versatility and establishes them as robust biocatalysts for producing structurally complex oxylipins.

Current challenges and outlook

Although bacterial LOXs hold significant potential due to their diverse dioxygenation and hydroperoxide isomerase activities, scaling up their use for large-scale applications remains challenging. Heterologous production of bacterial LOXs usually is performed in *Escherichia coli* (Andreou et al. 2008; Lu et al. 2013b; Neau et al. 2014; Sim et al. 2015; Newie et al. 2016; Qian et al. 2017). However, heterologous production in *E. coli* results in extensive formation of inclusion bodies (Vidal-Mas et al. 2005; Lu et al. 2013b) and production of enzymes with low iron loading (Andreou et al. 2010; Deschamps et al. 2016; Goloshchapova et al. 2018; Chrisnasari et al. 2024b). Various strategies, such as lowering temperature and inducer concentrations, have been employed to balance gene expression and protein folding efficiency (Andreou et al. 2008; An et al. 2015, 2018a; Pang et al. 2020; Chrisnasari et al. 2024b). Yet, these adjustments were not always successful.

To fully harness the potential of bacterial LOXs, a paradigm shift in expression systems and optimization strategies is essential. The recent successful overexpression of *Pseudomonas aeruginosa* LOX in the yeast *Komagataella phaffii* (formerly known as *Pichia pastoris*) offers a promising alternative (Hashem et al. 2020). This yeast system facilitates the secretion of LOX into the supernatant, significantly simplifying downstream processing compared to *E. coli*. However, key factors, such as the iron content of the expressed protein, remain unassessed. Therefore, further evaluation of the different expression systems is crucial to unlocking the full potential of bacterial LOXs for various applications.

In addition to production challenges, some bacterial LOXs have been reported to be thermally unstable (Busquets et al. 2004; Qian et al. 2017; Chrisnasari et al. 2024b). Similarly, low thermostability has also been reported for soybean mini-LOX, an enzyme that lacks the N-terminal beta-barrel domain found in native soybean LOX (Di Venere et al. 2003). The low stability of this mini-LOX is attributed to greater hydration compared to the native enzyme (Di Venere et al. 2003). As most bacterial LOXs have been predicted not to contain an N-terminal beta-barrel domain (Chrisnasari et al. 2022), it is possible that these enzymes are more hydrated and thus less stable. This finding highlights the urgent need for strategies to enhance the stability of bacterial LOXs. Rational protein engineering through mutagenesis, guided by computer-aided approaches such as FRESCO (Wijma et al. 2018) or computationally assisted structure-guided design (Chi et al. 2026), offers a viable solution. Techniques such as site-directed mutagenesis (Guo et al. 2014), deletion of the highly flexible region (Lu et al. 2014), and fusion with self-assembling amphipathic peptides (Lu et al. 2013a) have demonstrated success in improving the thermostability of bacterial LOXs.

Overcoming these challenges requires a multidisciplinary approach that integrates advanced expression systems, enhanced protein engineering, and novel stabilization techniques. Addressing these limitations will not only pave the way for the efficient large-scale production of bacterial LOXs, but also unlock their full potential in biotechnological applications. However, applying bacterial LOXs across various industries requires balancing the benefits of “green” production with the often higher costs of enzymatic biocatalysis. Therefore, LOXs are particularly well-suited for synthesizing high-value products, such as lipid mediators, where these advantages justify the investment.

Conclusions

Bacterial LOXs are promising biocatalysts for fatty acid oxyfunctionalization, utilizing regioselective dioxygenation and hydroperoxide isomerization activities. Strategies such as protein engineering and oxygen level modulation effectively modify substrate specificity, regioselectivity, and the preferred catalytic pathway. Multi-enzyme cascades incorporating LOXs facilitate the production of diverse flavors, fragrances, oleochemicals, and oxylipins. This review also highlights efficient pathways for synthesizing oxylipins via LOX-mediated double dioxygenation and combined dioxygenase-hydroperoxide isomerase activities. To fully realize their potential, advancements in expression systems and enzyme thermostability are essential. This review not only enhances our understanding of bacterial LOX enzymology but also provides a foundation for their broader adoption in sustainable and innovative biotechnological applications.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Competing interests The authors declare no competing interests.

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