EGYPTIAN JOURNAL OF BOTANY (EJBO)

Print ISSN: 0375-9237
Online ISSN: 2357-0350

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PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

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REVIEW ARTICLE

Cholesterol oxidase is a profound enzyme that recently gained much attention, especially for its numerous therapeutic and biotechnological applications, as a rapid and sensitive assay for cholesterol estimation in various clinical samples, by catalyzing the conversation of cholesterol into cholest-4-en-3-one and reducing oxygen to hydrogen peroxide. The immobilized enzyme has been commonly used as an electrode in the colorimetric biosensors of cholesterol estimation, with a high sensitivity and selectivity for arteriosclerosis diagnosis, preparation of the ketosteroids from the corresponding ones, and steroids analysis in food samples. This enzyme was partially purified and characterized from various microbial sources. Nevertheless, the availability, structural stability, and catalytic efficiency of the completely purified cholesterol oxidase remain the major challenges. So, the objective of this review was to give an insight into the different sources, biochemical and catalytic properties of cholesterol oxidase from the literature study, in addition to the possible approaches to increase its reusability and operative stability via different immobilization techniques.

Keywords: Cholesterol Oxidase; Bacterial sources; Biochemical properties; Catalytic efficiency

ARTICLE HISTORY
Submitted: February 17, 2025
Accepted: May 10, 2025

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DOI: 10.21608/ejbo.2025.358992.3183

EDITED BY: N. Khalil

INTRODUCTION

Cholesterol oxidase enzymes have diverse biotechnological applications, as rapid and sensitive assay cholesterol determination in food products, in addition to all the clinical samples (Fazaeli *et al*., 2018). Cholesterol oxidase-based colorimetric biosensor was implemented for rapid detection of cholesterol in serum (Ghosh *et al*., 2018). The researchers developed a cholesterol oxidase-based colorimetric biosensor for the rapid detection of cholesterol in serum, by immobilizing the enzyme on a gold electrode surface, that shows a very sensitive assay with higher selectivity. Cholesterol oxidase is widespread applications in clinical and industrial aspects for cholesterol assessment in arteriosclerosis diagnosis, steroids analysis in food samples (Kumari *et al.,* 2012). So, the objective of this review was to summarize the findings of molecular and biochemical properties of the cholesterol oxidase, as well as to address the potential application of this enzyme in various biotechnological industries.

Cholesterol oxidase catalytic mechanism

Cholesterol oxidase is a monomeric enzyme, which catalyzing the conversion of cholesterol into cholest-4-en-3-one and the reduction of oxygen to hydrogen peroxide via a two-step process; oxidation of cholesterol into cholest-5-en 3- one via a redox reaction in which FAD is the cofactor (Sakodinskaya and Ryabov, 2000, Caldinelli *et al*., 2005), followed by isomerization of cholest-5-en 3 one into cholest -4-en -3-one (Ahn and Sampson, 2004), as follows; Cholesterol oxidase catalyzes three chemical transformations, giving two redox equivalents that transferred to the oxidized flavin that becomes reduced, and the reduced one reacts with dioxygen to generate hydrogen peroxide. The third catalytic step involves isomerization of the double bond in the steroid ring (from D5-6 to D4-5) to form cholest-4-en-3-one. The selectivity of cholesterol oxidase for numerous substrates was derived from the cholestane skeleton as a critical step in enzyme kinetics (Kreit and Sampson, 2009). The oxidation of cholesterol by cholesterol oxidase is important in the metabolism of this lipid molecule by microorganisms, and has several potential applications in biotechnology, medicine, and food science (Wali *et al*., 2011). Pollegioni *et al.,* (2009) confirm the oxidation of cholesterol by oxidase, producing hydrogen peroxide that was enzymatically assessed by horseradish peroxidase via the oxidative coupling of 4-aminoantipyrine and phenol that can be measured as quinoneimine dye at λ_{500} nm (Cheillan *et al.,* 1989).

Sources of cholesterol oxidase

Endophytic bacteria usually live in the plant tissues without any visual symptoms of diseases on the plants, colonizing the intracellular space of the cell wall and xylem vessels and leaves (Zheng et al., 2022). Endophytic bacteria have been considered as a new source of diverse secondary metabolites with numerous bioactivities (Singh *et al.,* 2017 a, 2017b). Endophytic bacteria are the subject of numerous studies for their potential applications in agriculture, medicine, and biotechnology (Siddalingeshwara *et al.,* 2013, Kumar *et al*, 2014). Several types of endophytic

bacteria reported to produce cholesterol oxidase include *Bacillus cereus* (Khandelwal *et al*., 2015, Siddalingeshwara *et al.,* 2013), *Myobacterium, Rhodococcus, Brevibacterium, Streptomyces, Comamonas, Burkholderia, Pseudomonas*, and *Chromobacterium* (Wali *et al.,* 2011). Several Egyptian isolates of Actinomycets have been identified as cholesterol oxidase producers (Abd El-wahed *et al.,* 2021). *Actinomyces lavendulae* (Petrova *et al.,* 1979), *Arthrobacter simplex* (Liu *et al*., 1988) and *Arthrobacter* spp (Chen *et al.,* 2016) were identified as cholesterol oxidase producers. As well as, *Bacillus* spp (Rhee *et al.,* 2002) *Bacillus cereus* (Kuppusamy and Kumar, 2016), were reported as a producer of cholesterol oxidase. In addition, *Brevibacterium* sp*, Brevibacterium sterolium* (Motteran *et al*., 2001) and *Bordetella* sp (Lin *et al.,* 2010) were reported as potential producers of cholesterol oxidase. *Chromobacterium* sp, *Chryseobacterium* sp and *E. coli* were reported as cholesterol oxidase producers, with potential applications in clinical diagnostics and biotechnology (Doukyu *et al*., 2008, Reiss *et al.,* 2014, Fazaeli *et al.,* 2019). Kulkarni *et al.*, (2013) suggests that certain strains of *Lactobacillus* could modulate cholesterol metabolism, thus lowering the cholesterol levels in the body. Cholesterol can be used as a sole carbon source for growth of *Nocardia* sp and *Rhodococcus* sp (Kreit *et al*., 2017, Sojo *et al.* 2002, Lashkarian *et al.,* 2010). As well as *Streptoverticillium cholesterolicum* was reported as a potential cholesterol oxidase producer (Inouye *et al.* 1982). Cholesterol oxidase was purified and characterized from *Escherichia fergusonii* strain from local whey samples, and the enzyme productivity was optimized by the combined experimental study with the artificial neural network modeling (Khataee et al., 2025).

Cultural conditions for production of cholesterol oxidase by microorganisms

The microbial cultural conditions for cholesterol oxidase production from the different bacterial isolates were studied. Glycerol supplementation in the fermentation medium significantly increased the productivity of cholesterol oxidase by *Streptomyces* sp. by about 3.2-fold increase compared to control medium (Zhang *et al.,* 2014, Devi *et al.*, 2021). As well as Casein was reported to be strongly induces the productivity of cholesterol oxidase by *Streptomyces* sp (liu *et al.,* 2017a). Peptone and potato starch supplementation in the fermentation media strongly increase the enzyme productivity by *Bacillus* sp and *Streptomyces* sp (Zhang *et al*., 2019). Addition of cholesterol to the basal media of *E. hira*, strongly induce cholesterol oxidase production (Yehia *et al*., 2015*). Comamonas, Burkholderia, and Enterococcus* are only a few more Gram-positive and Gram-negative bacteria that have been widely studied (Vrielink and Ghisla, 2009, Yehia *et al*., 2015). Bacterial cholesterol oxidase producing isolates were isolated from waste of regional oil mill, soil and compost and identified as *Streptomyces* sp., *Arthrobacter* sp. and *Aspergillus* sp. In addition, *Enterococcus* sp, *Pseudomonas* sp., *Bacillus pumilus, Serratia marcescens* (Wali *et al.,* 2011), and *Streptomyces rochei* (Elsayed and Abd el-wahed, 2020), were reported as potential enzyme producers.

Purification, biochemical properties and crystal structures of cholesterol oxidase from bacteria

Cholesterol oxidase was commonly purified from different microorganisms using various chromatographic approaches especially ion exchange and gel filtration chromatography (Gao *et al.,* 2020). The purified enzyme showed reasonable activity and stability with a wide pH range, reaction temperature, and broad thermal stability. Adding Tween 80, Triton X-100 to the culture medium resulted in a significant increase in enzyme activity, in contrary to the inhibitory effect by the SDS (Gao et al., 2020). Chen *et al.* (2021) have explored the different purification methods for COX enzyme, including the use of ion exchange chromatography, ultrafiltration, and affinity chromatography, from *Bacillus subtilis*. The purified enzyme showed relatively higher activity and stability at a wide pH range and temperature conditions, that being suitable in the detection of cholesterol in food. Overall, the purity of cholesterol oxidase from endophytic bacteria has been assessed frequently through various methods, including the use of different chromatography techniques, optimization of culture conditions and the addition of surfactants to the medium (Li *et al.,* 2015). Acetone is commonly used as a precipitating agent for cholesterol oxidase, due to its high solubility in water, low toxicity to proteins with little deleterious effect on proteins and feasibility of removal by centrifugation (Bholay *et al*., 2013). Purification of cholesterol oxidase from *Nocardia rhodochrous, Brevibacterium* sp. and *Streptomyces* by ion-exchange chromatography was frequently reported (El-Sayed and Ali, 2011, Singh *et al.* 2016, Zarei *et al*, 2022). Cholesterol oxidase from *Burkholderia gladioli* was purified and immobilized on Calcium alginate, with a robust catalytic stability for cholesterol oxidation (El-Sayed et al., 2025). Its crystal

of cholesterol oxidase reveals a homodimer protein with each subunit containing a flavin adenine dinucleotide (FAD) cofactor that participates in the catalytic mechanism. The identity of catalytic residues and phylogenetic distribution of cholesterol oxidase is cleared. The biochemical properties of cholesterol oxidase from different microorganisms were summarized in Table 1.

Different approaches for improving the stability of cholesterol oxidase

Different approaches have been implemented to increase the structural stability and catalytic efficiency of the enzymes, such as carrier, covalent binding, polymer entrapment, and cross-linking (Bayraktar and Serilmez, 2011). The catalytic stability of enzymes was strongly enhanced with immobilization on nano-matrices, for their huge surface area and compact size. The reusability and stability of the immobilized enzymes are the major features for the enzyme-nanoconjugates (Ghosh *et al.,* 2018). The mesoporous cages and surface of MOF PCN-333(Al), which was employed as a colorimetric biosensor for cholesterol detection, were co-conjugated with cholesterol oxidase and HRP (Zhao *et al.,* 2019). Compound Tris (2,2-bipyridyl) dichloro-ruthenium (II) hexahydrate (Ru (bpy) 3Cl2) was present in Fe_3O_4&SiO_2-SiO_2-COX nanoparticles, was used as a sensing material, has a potential application in multi-parameter fiber optic biosensors (Huang *et al.,* 2015).

Hwang and Lee (2019) identified three primary methods for multi-enzyme immobilization: random co-immobilization, positional co-immobilization, and compartmentalization. Using enzymes like cholesterol esterase (ChEt)/oxidase, biosensor surfaces were modified to particularly increase the sensitivity towards biological moieties to detect cholesterol level in blood or human serums. Chitosan nanocomposites-based biosensors have been demonstrated to be more effective, and sensitive (Murugesan and Scheibel, 2021). The different approaches chemical modifications of enzyme via immobilization on different compatible biopolymers, such as dextran, Calcium alginate, Polyethylene glycol, chitosan, chitin, and cyclodextrin for L-methionine γ-lyase, arginase, L-arginine deiminase, and L-asparaginase were reported in our previous studies (El-Sayed et al., 2011, 2013, 2015a, b, 2022, 2023., 2024, 2025, Kebeish et al., 2016, Yassin et al., 2022).

Biotechnological applications of microbial cholesterol oxidases

Cholesterol oxidase is an enzyme that catalyzes the oxidation of cholesterol into cholest-4-en-3-one, which is an important reaction in various biotechnological applications. Immobilization of cholesterol oxidase can enhance its stability, reusability, and activity, making it more efficient for industrial applications, making this enzyme as a proper enzyme for industrial and clinical laboratory applications (Torkaman and Soudi, 2021). Cholesterol oxidase is used in measuring cholesterol in clinical samples, bioconversion of cholesterol and insecticidal activity against cotton weevil were explored (Doukyu and Aono *et al.,* 2001, El-Naggar and El-Shweihy, 2020a). There are some medical uses of cholesterol oxidase produced by endophytic bacteria that have been shown to effectively reduce cholesterol levels in animal models by converting cholesterol to cholest-4-en-3-one, that can be excreted from the body (Singh *et al.,* 2016, Shah and Khullar, 2017).

Cholesterol has received much attention recently for its role in carcinogenesis as reported from different studies (Silvente and Poirot, 2012). Cancer is an uncontrolled cell growth, resistance to apoptosis, in addition, deregulation of lipid metabolism is one of the emerging metabolic hallmarks in cancer development (Liu *et al.,* 2017b). *In vivo* study showed that combination therapy has a more powerful activity to combat the tumor cell growth than either cholesterol oxidase or Dox treated groups singly (Liu *et al.,* 2017b). Cholesterol oxidase treatment resulted in phosphorylation of JNK (c-Jun NH2-terminal kinase) and p38, downregulation of Bcl-2 (B-cell lymphoma/leukemia-2), upregulation of Bax, activating caspase-3 and cytochrome C likely via production of H_2O_2 along with cholesterol oxidation (Devi and Kanwar, 2017). So, cholesterol has an important role in cancer development (Ding *et al.,* 2019).

The clinical and experimental studies revealed that the high-cholesterol diet has a direct effect on cancer development, which is a critical branch of the cholesterol synthesis for breast cancer stem cell (CSC) maintenance. Cholesterol oxidase is used in the production of steroid hormones such as cortisol, aldosterone, and testosterone. It is used to convert cholesterol to cholest-4-en-3-one, which is a precursor for the synthesis of these hormones, and bile acid synthesis. Cholesterol oxidase has been used practically in producing of the precursors of hormonal

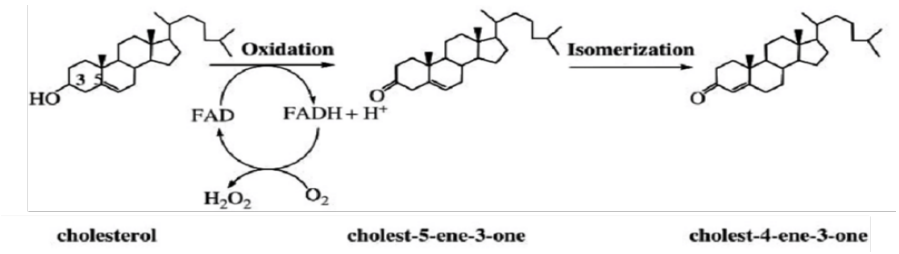

Figure 1. Schematic diagram of cholesterol oxidation and isomerization catalysed by cholesterol oxidase

Table 1. Biochemical properties of cholesterol oxidase from different microorganisms

Microorganism	Optimum pH	pH Stability	Optimum Temperature	Specific Activity	Molecular Subunit Structure (kDa)	References
Arthrobacter simplex	7.5	7.0-8.0	50	3.6	57	Liu et al., 1988
Bacillus subtilis	7.5	7.0-8.0	37	1.39	105	Kumari and Kanwar, 2016
Brevibacterium sterolicum	6.5	6.0-7.0	55	55.2	46.5	Fujishiro et al., 2002
Burkholderia cepaca ST-200	6.8-8.0	6.0-7.0	60	16.9	60	Doukyu N, Aono R (2001
Chryseobacterium gleum	6.75	6.2-7.4	35	15.5	60	Reiss et al., 2014
Corynebacterium cholesterolicum	7.0-7.5	6.5-7.9	40-42	-	57	Shirokane YNK, Mizusawa, 1977
Enterococcus hirae	7.8	7.0-8.0	40	124	60	Yehia et al., 2015
Streptomyces fradiae	7	6.0-7.0	70	-	60	Yazdi et al., 2001
Pseudomonas aeruginosa	7.0	6.0-7.0	70	11.6	59	Doukyu N, Nihei, 2015
Streptomyces aegyptia	7.0	6.0-7.0	37	15.631	46	El-Naggar et al., 2017
Streptomyces rimosus	7	6.4-7.6	40	-	-	Srivastava et al., 2018
Streptomyces virginiae	7.5	7.0-9.0	45	-	58.9	Li et al., 2010

steroids as one of the physiological functions of this enzyme (Kreit and Sampson, 2009, Hamed *et al.,* 2010). The enzyme has broad applications on the biosensor manufacturing, biotransformation of steroids, and polyene macrolide pimaricin synthesis (Devi and kanwar, 2017). Nanotechnology-based tools could be one of the most sophisticated approaches for the enzyme-based biosensors in clinical applications (Narwal *et al.,* 2019, Akanksha and Kavindra 2020).

HOx treatment inhibited phosphorylaon of Akt (protein kinase B) and ERK1/2 (extracellular signal regulated kinase 1/2) which was irreversible even aer cholesterol addion. Further studies indicated that Choletserol oxidase treatment also promoted the generaon of reacve oxygen species (These findings suggested that Choletserol oxidase leads to irreversible cell apoptosis by decreasing cholesterol content and increasing ROS level. This indicates that the microbial Cholesterol oxidase may be a promising candidate for a novel anti-tumor therapy [Liu et al 2014 ; Devi and Kanwar (2017)]. Liu J, Xian G, Li M, Zhang Y, Yang M, et al (2014) Cholesterol oxidase from Bordetella sp. promotes irreversible cell apoptosis in lung adenocarcinoma by cholesterol oxidaon. Cell Death and Disease 5: 1372 Liu J, Xian G, Li M, Zhang Y, Yang M, et al (2014) Cholesterol oxidase from Bordetella sp. promotes irreversible cell apoptosis in lung adenocarcinoma by cholesterol oxidaon. Cell Death and Disease 5: 1372 HOx treatment inhibited phosphorylaon of Akt (protein kinase B) and ERK1/2 (extracellular signal-regulated kinase 1/2) which was irreversible even aer cholesterol addion. Further studies indicated that Choletserol oxidase treatment also promoted the generaon of reacve oxygen species (ROS).

Cholesterol oxidase produced by endophytic bacteria has been shown to have antibacterial properties. It works by disrupting the bacterial cell membrane, leading to bacterial cell death (Vinothkumar and Annamalai, 2018). Cholesterol oxidase based biosensors can be used in cancer diagnosis and therapy (Kharitonov and Shumyantseva (2019). There is a substantial interest in the production of this enzyme, especially for their wide applications on assay of the total and free cholesterol (Hamed *et al.,* 2010). The enzyme has a crucial role in bacterial pathogenesis (Vrielnik, 2010), as a membrane-damaging factor of *R. equi,* an opportunistic human pathogen (Navas *et al.,* 2001). Cholesterol oxidase

was reported to be produced by pathogenic bacteria, *Rhodococcus* sp (Fernández *et al.* 2014), *Bordetella* sp (Lin *et al.* 2010) and *Mycobacteria* sp (Yao *et al*., 2013) for invading the host cells, by altering the structure of cholesterol of the cell membrane to cholesten-4-en-3-one. In addition, due to the frequency of cholesterol oxidase in bacteria and fungi, this enzyme has been considered as a possible target of antibiotics. The beta amyloids in Alzheimer's disease oxidize cholesterol producing 4-cholesten-3-one, mimicking the cholesterol oxidase activity (Sottero *et al.,* 2009). This enzyme involved transformation of sterols and nonsteroidal compounds (Ahire *et al.,* 2012), analysis of the membrane structure, and biosynthesis of polyene macrolide pimaricin (Doukyu 2009). Another use of the enzyme is in the microanalysis of steroids in food specimens for determining the steric configuration of 3-ketosteroids from their corresponding 3β-hydroxysteroids (Kuppusamy and Kumar, 2016). Cholesterol oxidase has been immobilized on chitin, a natural biopolymer (Yazdi *et al.,* 2001a), upon cross linking by glutaraldehyde, displaying higher thermal stability, and reusability, and retained more than 90% of its initial activity after 10 cycles of reuse. Immobilization of cholesterol oxidase on magnetic nanoparticles by covalent bonding to the surface of nanoparticles with a bifunctional link, with affordability of separation of the enzyme from the reaction mixture, which is important for industrial applications (Puri *et al*., 2019). The conjugated enzyme showed higher thermal and pH stability and retained more than 80% of its initial activity after 10 cycles of reuse. Cholesterol oxidase conjugated with chitosan magnetite was able to oxidize up to 90% of a cholesterol substrate, the enzyme reacted with chitosan-aminated magnetite nanoparticles (Saravanan and Gomathi 2016, Senthilkumar and Gomathi 2018). Poly-Ethylene Glycol and decorated polystyrene nanoparticles were used to immobilize this enzyme for biosensors manufacturing (Ghosh *et al.,* 2018, Perdani *et al.,* 2020).

Conclusion and future directions of cholesterol oxidase applications

The biochemical and kinetic properties of cholesterol oxidase from the different bacterial sources, as well as the different immobilization approaches to enhance the enzyme catalytic stability, operative stability and reusability, were reported. However, catalytic efficiency and stability were the main challenge that halts the further applications of this enzyme in various biotechnological aspects. So, screening for this enzyme from different extraordinary bacterial or fungal sources, such as thermophilic, barophilic conditions, could be a new approach to explore an enzyme with unique biochemical properties and affordable catalytic efficiency. In addition, cholesterol oxidase immobilization on novel magnetic nanoparticles, or nanopolymers with little chemical interactions, could be an alternative tool to get a highly active enzyme with numerous and limitless operative stability of this enzyme.

REFERENCES

Ahire JJ, Bhat AA, Thakare JM, Pawar PB, Zope DG, Jain RM, Chaudhari BL. (2012). Cholesterol assimilation and biotransformation by *Lactobacillus helveticus*. Biotechnology Letters, 34, 103–107.

Ahn K.W., Sampson N.S. (2004). Cholesterol oxidase senses subtle changes in lipid bilayer structure. Biochemistry; 43:827–836.

Akanksha V. M., and Kavindra K. K. (2020). Microbial Cholesterol Oxidase: Industrial Applications. Microbial Enzymes: Roles and Applications in Industries . 11: 303-317

Bayraktar H., Serilmez M. (2011). Immobilization and stabilization of α-galactosidase on Sepabeads EC-EA and EC-HA. Int J Biol Macromolec. 49: 855-860.

Bholay A. D., Gadekar D. J., Sawant S. K., Sonawane S. M. Original 2013: research article bacterial extracellular cholesterol oxidase and its pharmaceutical perspectives. *Intern J Current Microbiol. Appl. Sciences* , 2(3):19–28.

Caldinelli L, Iametti S, Barbiroli A et al (2005) Dissecting the structural determinants of the stability of cholesterol oxidase containing covalently bound flavin. J Biol Chem., 280: 22572–22581.

Cheillan F., Lafont H., Termine E., Hamann Y. and Lesgards G. (1989). Comparative study of methods for measuring cholesterol in biological fluids. Lipids 24, 224– 228.

Chen S., Gao B. and Li H. (2021). Effects of surfactants on the production and purification of cholesterol oxidase from *Streptomyces* sp. J Bioscien Bioengin. 131(1): 96-102

Chen Y.R., Huang H.H., Cheng Y.F., Tang T.Y. and Liu W.H. (2016). Expression of a cholesterol oxidase gene from *Arthrobacter simplex* in *Escherichia coli* and *Pichia pastoris*. Enzyme Microb Technol 39: 854-860.

Devi S. and Kanwar S. (2017). Cholesterol Oxidase: Source, Properties and Applications. Insights Enzyme Res., 1,1: 1-12

Devi S., Chauhan V. and Kanwar S. (2021). Optimization of culture conditions for extracellular cholesterol oxidase production from *Castellaniella* sp. for cholesterol conversion to 4-cholesten-3-one. Research J Biotechnol. 16: 15-25.

Doukyu N, Aono R (2001) cloning, sequence analysis and expression of a gene encoding an organic solvent and detergent-tolerant cholesterol oxidase of Burkholderia

cepacia strain ST200. Appl. Microbiol Biotechnol 57: 146-152.

Doukyu N, Nihei S (2015) Cholesterol oxidase with high catalytic activity from *Pseudomonas aeruginosa*: Screening, molecular genetic analysis, expression and characterization. J Bioscience Bioeng 120: 24-30.

Doukyu N, Shibata K, Ogino H, Sagermann M (2008). Purification and characterization of *Chromobacterium sp*. DS-1 cholesterol oxidase with thermal, organic solvent, and detergent tolerance. Appl Microbiol Biotechnol, 80(1):59–70.

Doukyu N. (2009) Characteristics and biotechnological applications of microbial cholesterol oxidases. Applied microbiology and biotechnology 83:825–837.

Doukyu N., and Aono R. (2001). Cloning, sequence analysis and expression of a gene encoding an organic solvent- and detergent-tolerant cholesterol oxidase of *Burkholderia cepacia* strain ST-200. Appl. Microbiol.

El-Naggar N, E and. El-Shweihy N.M. (2020). Identification of cholesterol-assimilating actinomycetes strain and application of statistical modeling approaches for improvement of cholesterol oxidase production by *Streptomyces anulatus* strain. BMC Microbiology, 20:86

El-Sayed. A. M. M. and Ali R. A. M. (2011). Purification and characterization of cholesterol oxidase produced by a *Streptomyces sp*.," World J. Microbiol. Biotechnol. 27: 8,1911-1918.

El-Sayed ASA, Shindia AA, El-Badry WM, Mostafa AA, A Al-Ghanayem A, Rady AM. (2025). Purification and Immobilization of *Burkholderia gladioli* Cholesterol Oxidase on Calcium Alginate, with Robust Catalytic Stability for Cholesterol Oxidation *In Vitro*. Curr Microbiol. 2025 Feb 5;82(3):119.

El-Sayed A.S.A., Shindia A., Emam E., Labib M., El-Deen E.N., Seadawy M.G., Yassin M.A. (2024a). Aspergillus flavipes L-methionine γ-lyase-β-cyclodextrin conjugates with improved stability, catalytic efficiency and anticancer activity. Scientific Reports, 14 (1), art. no. 27715,

El-Sayed A.S.A., Elghamry H.N., Yassin M.A. (2024b). Biochemical Characterization of Thermostable Acrylamide Amidohydrolase from Aspergillus fumigatus with Potential Activity for Acrylamide Degradation in Various Food Products. Current Microbiology, 81 (1), art. no. 30

El-Sayed A.S.A., Rady A.M., Mohamed H.T., Zein N., Yassin M.A., Mohamed N.Z., Hassan A., Amer M.M., El-Sharakawy R., El-Sharkawy A.A., El-Sayed N., Ali M.G. (2023). Aspergillus Niger thermostable Cytosine deaminase-dextran conjugates with enhanced structure stability, proteolytic resistance, and Antiproliferative activity. BMC Microbiology, 23 (1), art. no. 9.

El-Sayed A.S.A., Shindia A.A., Zeid A.A.A., Yassin A.M., Sitohy M.Z., Sitohy B. (2019). Aspergillus nidulans thermostable arginine deiminase-Dextran conjugates with enhanced molecular stability, proteolytic resistance, pharmacokinetic properties and anticancer activity. Enzyme and Microbial Technology, 131, art. no. 109432.

El-Sayed A.S.A., Yassin M.A., Ali G.S. (2015a). Transcriptional and proteomic profiling of Aspergillus flavipes in response to sulfur starvation. PLoS ONE, 10 (12), art. no. e0144304.

El-Sayed A.S.A., Yassin M.A., Ibrahim H. (2015b). Coimmobilization of l -methioninase and glutamate dehydrogenase: Novel approach for l -homoalanine synthesis. Biotechnology and Applied Biochemistry, 62 (4), pp. 514 – 522.

El-Sayed A.S., Shindia A.A. (2011). Characterization and immobilization of purified Aspergillus flavipes L-methioninase: Continuous production of methanethiol. Journal of Applied Microbiology, 111 (1), pp. 54 – 69.

El-Sayed A.S., Khalaf S.A., Aziz H.A. (2013). Characterization of homocysteine γ-lyase from submerged and solid cultures of Aspergillus fumigatus ASH (JX006238). Journal of Microbiology and Biotechnology, 23 (4), pp. 499 – 510.

Fazaeli A., Golestani A., Lakzaei M., Rasi-Varaei S.S., Aminian M. (2019). Expression optimization, purification, and functional characterization of cholesterol oxidase from *Chromobacterium s*p. DS1. PLoS ONE, 14(2):1-15.

Fujishiro K, Uchida H, Shimokawa K, Nakano M, Sano F, (2002). Purification and properties of a new *Brevibacterium sterolicum* cholesterol oxidase produced by *E. coli* MM294/ pnH10. FEMS Microbiol Lett 215: 243-248

Gao J., Wang Y., Chen X. (2020). Purification and characterization of cholesterol oxidase from *Streptomyces griseus*. Journal of Microbiology and Biotechnology, 30(2): 271-277

Ghosh S., Ahmad R. and Khare S.K. (2018). Immobilization of Cholesterol Oxidase: An Overview. The Open Biotechnology Journal, 12:176-188

Huang Z., Bao Y.Y., Yuan T.T., Wang G.X., He L.Y., Sheng X.F. (2015). *Arthrobacter nanjingensis* sp. nov., a mineral-weathering bacterium isolated from forest soil. Int J Syst Evol Microbiol; 65:365-369.

Hwang E. T., and Lee S. (2019). Multi enzymatic cascade reactions via enzyme complex by immobilization. ACS Catal. 9, 4402–4425.

Inouye Y., Taguchi, K., Fuji A., Ishimaru K., Nakamura S., Nomi R. (1982). Purification and characterization of extracellular 3b-hydroxysteroid oxidase produced by *Streptoverticillium cholesterolicum*. Chem Pharm Bull 30, 951–958.

Kebeish R., El-Sayed A., Fahmy H., Abdel-Ghany A. (2016). Molecular cloning, biochemical characterization, and antitumor properties of a novel L-asparaginase from Synechococcus elongatus PCC6803. Biochemistry (Moscow), 81 (10), pp. 1173 – 1181.

Khandelwal S. R., Sharma A., Gupta, N. and Gupta, V. K. (2015). Isolation and characterization of cholesterol

oxidase producing endophytic bacteria from oilseed crops. J. Appl. Microbiol., 118(3), 855-86.

Khataee, S., Dehghan, G., Rashtbari, S. Marefat, A., Jamei, S., Farzi-Khajeh, H (2025). Optimized extraction and kinetic study of cholesterol oxidase from a newly isolated Escherichia fergusonii strain from local whey samples: insights through a combined experimental study and artificial neural network modeling. BMC Microbiol 25, 32.

Kharitonov A. B. and Shumyantseva V. V. (2019). Cholesterol oxidase-based biosensors in cancer diagnosis and therapy. Biosensors and bioelectronics, 137,211-221

Kim HJ. and Kim J.H. (2017). Cholesterol oxidase: biological functions and biotechnological applications. J. Microbiol. Biotechnol. 27(11): 1873-1884

Kreit J. and Sampson N.S. (2009). Cholesterol oxidase: physiological functions. FEBS Journal 23, 1742.

Kulkarni N. S., Lokhande A. P., Pachori R. R., Agrawal P. N. and Dalal J. M. (2013). Screening of the Cholesterol Degrading Bacteria from Cow's Milk Current Research in Microbiology and Biotechnology. 1:3: 92-94.

Kumar P., Mahajan S., Singh D., Kumari R., Kumar A., Singh A., Kumar V., Kamal S., Nigam A. and Saxena A.K. (2014). Cholesterol oxidase producing novel endophytic bacterium *Enterobacter sp*. PDN3 of pharmaceutical significance: isolation and molecular identification with partial characterization of enzyme. PLoS One. Oct 13;9(10):109688

Kumari L. and Kanwar S.S. (2016). Purification and Characterization of an Extracellular Cholesterol Oxidase of *Bacillus subtilis* Isolated from Tiger Excreta. Appl Biochem. Biotechnol., 178: 353-367.

Kuppusamy A. and Kumar K. V., (2016): "Optimization of cholesterol oxidase production and 16S rRNA partial sequence of *Bacillus cereus* strain KAVK4 isolated from butter," J Appl. Pharmac. Science. 6: 7; 61–66,

Lashkarian H., Raheb J., Shahzamani K., Shahbani H. Shamsara M. (2010). Extracellular cholesterol oxidase from *Rhodococcus* sp.: isolation and molecular characterization. Iran Biomed J., 14(1–2):49–5

Li Y., Li X., Wang B. and Dong, Y. (2015). Enhanced production of extracellular cholesterol oxidase by *Rhodococcus* sp. AS2 using response surface methodology. Biochemical Engineering Journal, 96, 15-22.

Li, B.; Wang, W.; Wang, F.; Wei, D. Cholesterol oxidase ChoL is a critical enzyme that catalyzes the conversion of diosgenin to 4-ene-3-keto steroids in Streptomyces virginiae IBL-14 (2010), Appl. Microbiol. Biotechnol., 85, 1831-1838.

Lin Y.L., Fu J.A. and Song X. (2010). Purification and characterization of an extracellular cholesterol oxidase from a *Bordetella* sp. Process Biochem., 45: 1563-1569.

Liu C., Wang Y., Li, Z. and Zhou Z. (2017a). Optimization of casein as an inducer for cholesterol oxidase production by *Streptomyces sp*. CH7. Journal of Microbiology and Biotechnology, 27(3), 478-485.

Liu Q. Luo Q., Halim A. and Song G. (2017b). Targeting lipid metabolism of cancer cells: a promising therapeutic strategy for cancer. Cancer Lett. 2;401: 39–45.

Liu W. H., Meng M. H. and Chen K. S. (1988). "Purification and Some Properties of Cholesterol Oxidases Produced by an Inducible and a Constitutive Mutant of *Arthrobacter simplex*," Agricultural and Biological Chemistry, Vol. 52, No. 2, pp. 4130-4138.

Mendes M.V., Recio E. and Antón N. (2007): Cholesterol oxidases act as signaling proteins for the biosynthesis of the polyene macrolide pimaricin. Chem Biol; 14(3): 279-290

Motteran L., M. S. Pilone G. Molla S. Ghisla S. and L. Pollegioni (2001). Cholesterol oxidase from *Brevibacterium sterolicum* the relationship between covalent Flavinylation and redox properties. The Journal of Biological Chemistry. Vol. 276: 18024-18030

Murugesan S. and Scheibel T. (2021). Chitosan-based nanocomposites for medical applications. J Polym Sci., 59:1610–1642.

Narwal V., Deswal R., Batra B., Kalra V. and Hooda R. (2019). Cholesterol biosensors: a review Steroids, 143: 1-6.

Navas J., Gonzalez-Zorn B., Ladron N., Garrido P. and Vazquez-Boland J.A. (2001). Identification and mutagenesis by allelic exchange of choE, encoding a cholesterol oxidase from the intracellular pathogen *Rhodococcus equi*. J Bacteriol 183: 4796– 4805

Perdani M. S., Juliansyah M. D., Putri D.N., Utami T. S., Hudaya C., Yohda M. and Hermansyah H. (2020). Immobilization of Cholesterol Oxidase in Chitosan Magnetite Material for Biosensor Application: International Journal of Technology 11(4): 754-763.

Petrova Y. A., Podsukhina G. M., Dikun T. A. and Selezneva A. A., (1979). "Conditions of Isolation of Cholesterol Oxidase from *Actinomy.es lavendulae mycelium*," Applied Biochemistry and Microbiology. 15:2; 125-128.

Pollegioni L, Piubelli L. and Gianluca M. (2009). Cholesterol oxidase: biotechnological applications. FEBS Journal. 276: 6857–6870.

Pollegioni L., Wels G., Pilone M.S. and Ghisla S. (1999): Kinetic mechanisms of cholesterol oxidase from *Streptomyces hygroscopicus and Brevibacterium sterolicum*. Eur J Biochem 264, 140–151.

Puri M. and Kaur I. P. (2018). Cholesterol oxidase: sources, properties, applications, and relevance in lipid analysis. Critical reviews in food science and nutrition, 58(9), 1483-1499

Puri M., Sharma D. and Barrow C. J. (2019). Biotechnological applications of cholesterol oxidases: an overview. Critical Reviews in Biotechnology, 38(5), 745-760

Reiss R., Faccio G., Thony-Meyer L. and Richter M. (2014). Cloning, expression and biochemical characterization of the cholesterol oxidase Cg ChoA from *Chryseobacterium gleum*. BMC Biotechnol., 14: 4.6.

Sakodinskaya I. K. and Ryabov A. D., (2000): Crown ether activates cholesterol oxidase in low water media. Biotechnology Letters, 22, 2, 173–176685

Saravanan, D., & Gomathi, D. (2016). Molecular cloning, expression and characterization of cholesterol oxidase gene from Pseudomonas aeruginosa. Microbial Pathogenesis, 96, 77-83.

Senthilkumar S., and Gomathi D. (2018). Molecular cloning, expression and characterization of cholesterol oxidase gene from *Streptomyces* sp. CNQ-509. Biocatalysis and Agricultural Biotechnology, 13, 255-262.

Shah N., and Khullar M. (2017). Cholesterol oxidase: sources, characteristics, and analytical applications. Critical reviews in food science and nutrition, 57(10), 2155-2168.

Shirokane YNK, Mizusawa K (1977) Purification and some properties of an extracellular 3-hydroxysteroid oxidase produced by *Corynebacterium cholesterolicum*. J Ferm Technol 55: 337-345.

Siddalingeshwara K.G., Jayaraman G., Muthuraj M., Sivakumar N., Uthandi S. and Ponnusami V. (2013). Isolation and characterization of cholesterol oxidase producing *Bacillus cereus* strain from endophytic bacteria of a medicinal plant. Biotechnol. Letters, 35(4), 563-570

Silvente P. and Poirot M. (2012). Cholesterol metabolism and cancer: the good, the bad and the ugly. Current Opinion in Pharmacology, 12, 673-676

Singh A. K., Singh S. K., and Upadhyay R. (2016). Purification and characterization of cholesterol oxidase from *Pseudomonas aeruginosa*. Current Microbiology, 73(2), 180-186

Singh R. P., Shukla, P., & Singh, R. P. (2017): Cholesterol oxidase producing endophytic bacteria: a novel approach for biotransformation of phytosterols. Biocatalysis and Agricultural Biotechnology, 9, 89-95.

Sojo M.M., Bru, R. R. and Garcia, F. F. (2002): *Rhodococcus erythropolis* as a suitable source of cholesterol oxidase: cell-linked and extracellular enzyme synthesis, purification and concentration. BMC Microbiol 2, 3.

Sottero B, Gamba P, Gargiulo S, Leonarduzzi G & Poli G (2009). Cholesterol oxidation products and disease: an emerging topic of interest in medicinal chemistry. Curr Med Chem 16, –705.

Srivastava, A.; Singh, V.; Haque, S.; Pandey, S.; Mishra, M.; Jawed, A.; Shukla, P.K.; Singh, P.K.; Tripathi, C.K.M. Response surface methodology-genetic algorithm based medium optimization, purification, and characterization of cholesterol oxidase from *Streptomyces rimosus* (2018), Sci. Rep., 8, 10913 .

Torkaman S., and Soudi, M. R. (2021). Cholesterol oxidase: A review of biocatalytic properties and applications in various industries. J. Mol Catalysis B: Enzymatic, 183, 26-38

Vinothkumar K., and Annamalai A. (2018). Antibacterial activity of cholesterol oxidase produced by endophytic bacteria from medicinal plants. International journal of current microbiology and applied sciences, 7(3), 1223-1235

Vrielink A. and Ghisla. S. (2009). Cholesterol oxidase: biochemistry and structural features FEBS Journal; 276: 6826–6843.

Wali PV, Srivastava A. and Tripathi C KM. (2011). Purification and characterization of the enzyme cholesterol oxidase from a new isolate *Streptomyces* sp. Appl Biochem Biotechnol 165, 1414–1426.

Xu k., Chen X., Zheng R. and Zheng Y. (2020). Immobilization of Multi-Enzymes on Support Materials for Efficient Biocatalysis Bioeng. Biotechnol., 30: 201-214

Yao Y., Tang H., Ren H., Yu H., Wang L., and Zhang W. (2013). Iron (II)-dependent dioxygenase and N-formylamide deformylase catalyze the reactions from 5-hydroxy-2-pyridone to maleamate. Sci. Rep., 3: 3235.

Yassin M.A., Shindia A., Labib M., Soud M., El-Sayed A.S.A. (2022). Thermostable Chitosan-L-Asparaginase conjugate from Aspergillus fumigatus is a novel structurally stable composite for abolishing acrylamide formation in French fried potatoes. LWT, 162, art. no. 113494,

Yazdi M.T., Malekzadeh F., Zarrini G., Faramarzi M.A., Kamranpour N. (2001). Production of cholesterol oxidase by a newly isolated *Rhodococcus sp*. World J Microbiol Biotechnol., 17: 731-737.

Yazdi MT, Zahraei M, Aghaepour K, Kuma-ranpour N (2001). Purification and Partial Characterization of Cholesterol Oxidase from *Streptomyces fradiae*. Enzyme Microb Technol 185: 410-414.

Yehia H. M., Hassanein W. A. and Ibraheim S. M. (2015): Purification and characterization of the extracellular cholesterol oxidase enzyme from *Enterococcus hirae*. *BMC Microbiol.,* 15–178

Zhang X., Li H., Zhang D., and Xu Y. (2014). Optimization of cholesterol oxidase production by *Streptomyces sp. S27* using response surface methodology. Bioresource Technology, 152, 545-550.

Zhang X., Li H., Zhang D., and Xu Y. (2019). Production of cholesterol oxidase from *Streptomyces sp. S9* using potato starch as an inducer. Biochemical Engineering Journal, 144, 72-78.

Zhao M., Li, Y., Ma, X., Xia, M., and Zhang, Y. (2019). Adsorption of cholesterol oxidase and entrapment of horseradish peroxidase in metal-organic frameworks for the colorimetric biosensing of cholesterol. Talanta 200, 293–299

Zheng, Y., Wang, C., Liu, W., Lu, G., and Shen, Q. (2022). Advances in the identification and application of endophytic bacteria. J. Agricultural Food Chem., 70:3: 742-754.