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Microbial Catalase as a Biotechnological Tool: From Diagnostic Enzyme to Therapeutic Target

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Abstract

Catalase is a haem-containing antioxidant enzyme that decomposes hydrogen peroxide (H_2O_2) into water and oxygen and is produced by most aerobic and facultative anaerobic bacteria. This review summarizes the roles of microbial catalase in bacterial survival and pathogenicity, its biotechnological and therapeutic applications, and its use in the identification and differentiation of bacteria. By detoxifying H_2O_2 generated during aerobic respiration or encountered from exogenous sources, catalase protects bacteria against oxidative damage, contributes to resistance to antiseptics, helps pathogens to evade phagocytic killing, and allows catalase-producing species to compete with H_2O_2-producing bacteria. Beyond these biological functions, microbial catalase is applied in the cosmetic, food, textile, pharmaceutical, agricultural, and environmental sectors, and serves as an auxiliary or reporter enzyme in biosensors for the detection of H_2O_2, glucose, and pathogenic bacteria. Catalase is also relevant to therapy, as illustrated by the activation of the anti-tubercular prodrug isoniazid by the catalase-peroxidase of *Mycobacterium tuberculosis*. In clinical microbiology, the catalase test remains a simple, rapid, and inexpensive presumptive test, for example, for differentiating *Staphylococcus* from *Streptococcus* species; however, its accuracy depends on the reagent concentration and quality, the culture medium, the inoculating tool, the age of the colonies, and the order of the tests. Understanding both the functions and the technical limitations of catalase is therefore essential for its effective use as a diagnostic enzyme and as a potential therapeutic target.

1. Introduction

Certain microorganisms, such as bacteria, and fungi produce catalase enzymes. Bacteria are prokaryotic organisms that lack a membrane-bound nucleus and other organelles, such as mitochondria, the endoplasmic reticulum, and the Golgi apparatus [1]. These single-celled microorganisms play a vital role in the ecosystems of our planet [2]. Like eukaryotic cells, they carry out biochemical activities [3]: they grow and reproduce, synthesize macromolecules (anabolism), and break down compounds (catabolism) to produce energy in the form of adenosine triphosphate (ATP). Bacteria possess various structural components and virulence factors that contribute to their survival and to their resistance to extreme environmental conditions [4]. Many bacteria share similar phenotypic characteristics and cannot be differentiated from one another on that basis alone. Molecular variations, the production of certain enzymes, and the ability to degrade particular organic compounds help to distinguish them, and catalase is one of these enzymes [5]. Therefore, the present review aims to provide a comprehensive overview of microbial catalase, highlighting its roles in microbial survival, oxidative stress resistance, and pathogenicity. It further explores its diverse industrial, biotechnological, pharmaceutical, and medicinal applications, with particular emphasis on its diagnostic applications in microbial identification and differentiation, as well as its therapeutic potential, particularly in prodrug activation and the development of targeted antimicrobial strategies.

2. Methodology

2.1. Data Collection and Search Strategy

This review was conducted through searches of major scientific databases, including PubMed, Scopus, Google Scholar, and Web of Science. The search was performed for articles and books published up to May 2026. The searh covered publications addressing the biochemical and molecular characteristics of microbial catalase, its roles in microbial survival, oxidative stress resistance, and pathogenicity, as well as its diagnostic, industrial, biotechnological, pharmaceutical, and medicinal applications. Boolean operators (AND, OR) were used for each database. The combined relevant keywords and key search terms were "microbial catalase", "bacterial catalase", "fungal catalase", "catalase-peroxidase", "catalase activity", "oxidative stress", "microbial survival", "microbial pathogenicity", "bacterial identification", "diagnostic microbiology", "industrial applications", "biotechnology", "prodrug activation", and "therapeutic applications".

2.2. Selection Criteria

The peer-reviewed original research, reviews, or books written in English that focused on bacterial or fungal catalase, with emphasis on its roles in microbial survival and pathogenesis and its applications in industrial and biotechnological applications, pharmaceutical and medicinal applications, and diagnostic applications were included in this review. Duplicate records or publications, those unrelated to the review objectives, and those focused exclusively on catalases produced by humans, other mammals, or plants without addressing microbial catalase were excluded.

2.3. Data Extraction and Synthesis

The selected publications were categorized into three main domains: the role of microbial catalase in oxidative stress defense, microbial pathogenicity, and applications in industry and biotechnology, pharmaceuticals and medicine, and diagnosis and identification of microbes. For the diagnostic applications, particular emphasis is placed on the procedures and limitations of catalase tests.

3. Catalase and Microbial Survival

3.1. Protection Against Reactive Oxygen Species

Microbes produce ATP by breaking down organic or inorganic compounds through a series of biochemical reactions, depending on whether or not they require oxygen (O_2) [6]. In the presence of oxygen, most obligate aerobic and facultative anaerobic bacteria completely oxidize organic compounds such as glucose to release energy and produce a large amount of ATP, with molecular O_2 serving as the terminal electron acceptor [7]. In obligate aerobes, which cannot live in the absence of O_2, aerobic respiration is the only source of energy [8]. In the absence of O_2, energy is obtained by fermentation or by anaerobic respiration, in which compounds such as sulfate (SO_4^{2-}), nitrate (NO_3^-), or sulfur (S) serve as terminal electron acceptors in obligate anaerobes [9]. These organisms live in O_2-free environments, where O_2 is toxic to strict anaerobes [10]. Facultative anaerobes can do both and can grow in either the

presence or the absence of O_2 [4]. The breakdown of glucose, called glycolysis, occurs in the presence or absence of O_2 and produces pyruvate through a series of reactions [11]. In aerobic respiration, pyruvate enters the tricarboxylic acid cycle (citric acid or Krebs cycle) and the electron transport chain to produce energy, carbon dioxide (CO_2), and water (H_2O) [12]. O_2 is used in oxidation-reduction reactions as the terminal electron acceptor of the electron transport chain [13]. In bacteria, glycolysis and the Krebs cycle occur in the cytoplasm, whereas the electron transport chain is located in the cytoplasmic membrane [14]. Because bacteria lack mitochondria, the cell membrane serves as their site of energy generation [4].

One disadvantage of normal aerobic respiration is the production of compounds that are extremely toxic to the bacterial cell [15]. These compounds are called reactive oxygen species (ROS) and include superoxide (O_2^-), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2) [16]. In addition, H_2O_2 that accumulates inside the bacterial cell can react with iron to produce more hydroxyl radicals [17]. ROS damage bacterial cells by breaking down intracellular macromolecules, such as nucleic acids, sugars, proteins, and lipids, eventually leading to cell death [18]. H_2O_2 is a strong oxidizing agent that inhibits the activity of cellular enzymes by oxidizing their sulfhydryl groups [19]. Protection against these harmful ROS is found in most obligate aerobic and facultative anaerobic bacteria, which produce a tetrameric haemoprotein enzyme called catalase (CAT), consisting of four polypeptide chains and a haem group (iron and porphyrin) (Table 1) [20]. Catalase acts together with other enzymes, such as superoxide dismutase (SOD), to convert ROS into harmless by-products [21]. The process begins with SOD, which converts superoxide radicals into hydrogen peroxide; catalase then completes the detoxification by decomposing cellular H_2O_2 into water and gaseous oxygen (Fig. 1) [22]. Catalase thus acts as a scavenger of H_2O_2 in the cell [23].

All strict anaerobic bacteria either cannot produce these protective enzymes or produce them at very low concentrations; therefore, they cannot tolerate O_2 and are killed by the toxic ROS generated during aerobic respiration [24]. This explains why these bacteria can grow only in the absence of O_2 [9]. They use fermentation to produce ATP, in which organic molecules serve as electron acceptors instead of O_2, thereby avoiding aerobic respiration and its harmful toxic by-products [25].

Some facultative anaerobes, such as *Streptococcus pyogenes* (group A *Streptococcus*), cannot produce catalase but can still grow and survive in the presence of oxygen and harmful ROS. They detoxify ROS using other enzymes, such as peroxidase, SOD, and nicotinamide adenine dinucleotide (NADH) oxidase [26, 27].

3.2. Resistance to Antiseptics

Hydrogen peroxide is one of the most common household topical antiseptics and is used to kill bacteria on human skin when cleaning small surgical wounds and rinsing scrapes and abrasions [28]. Catalase-producing bacteria may limit the toxic activity of this antiseptic [4].

4. Catalase and Microbial Pathogenicity

Protection against the harmful ROS generated by aerobic respiration allows bacteria to survive and to use other virulence factors to cause disease in humans [29].

4.1. Antiphagocytic Agent

Some microbes use catalase as an antiphagocytic agent to protect themselves against intracellular killing by the oxidative burst, particularly by ROS such as H_2O_2 (Table 1). For example, *Staphylococcus aureus* produces CAT, which is required for its survival and persistence [30], and *Acinetobacter* produces intracellular catalase-peroxidase (KatG) and catalase HPII (KatE), which confer resistance to H_2O_2 [31]. KatA produced by *Proteus mirabilis* is critical for biofilm formation [32]. Fungi such as *Histoplasma capsulatum*, *Paracoccidioides*, *Candida albicans*, and *Aspergillus* also possess catalases that detoxify H_2O_2 by degrading it into water and oxygen [33–36]. *H. capsulatum* expresses two catalases, CatB and CatP [37].

4.2. Competitive Agent

Some bacteria, such as *Streptococcus pyogenes* and *Streptococcus pneumoniae*, produce H_2O_2 to inhibit the colonization and growth of other bacteria in the upper respiratory tract, such as some species of *Haemophilus*, *Neisseria*, and *Moraxella* [38]. These H_2O_2-producing bacteria protect themselves against the harmful action of H_2O_2 by means of peroxidases [39]. H_2O_2 is also used as a virulence factor for competition, colonization, and spread through damage to host tissues [40]. Catalase-producing bacteria, such as *S. aureus*, can grow and compete with H_2O_2-producing bacteria because the enzyme detoxifies the harmful effect of H_2O_2 (Table 1) [41, 42]. The roles of catalase in bacterial survival and pathogenicity are summarized in Table 1.

Table 1
Summary of the roles of the catalase enzyme in bacterial survival and pathogenicity.

Category	Role	Mechanism
Microbial survival	Anti-ROS agent	Catalase detoxifies the bactericidal activity of H_2O_2 that is produced either by aerobic respiration or from exogenous sources [21].
	Resistance to antiseptics	The enzyme limits the toxic action of H_2O_2 used as an antiseptic [4].
Pathogenicity	Antiphagocytic agent	It helps bacteria to evade the immune response by preventing intracellular killing [30].
	Competitive agent	Catalase-producing bacteria can compete with bacteria that produce H_2O_2 [41].

5. Applications of Microbial Catalases

5.1. Cosmetic Industry

Catalase is used in cosmetic formulations in combination with other antioxidants [43].

CAT has also emerged in the personal care industry because of its highly efficient antioxidant properties. It is used in anti-aging formulations, in which it mitigates oxidative stress from environmental pollutants, such as ultraviolet (UV) radiation and ROS, to protect skin collagen and elastin structures, and it has an anti-inflammatory effect against skin irritation (Table 2) [44]. It is also combined with mild peroxide in facial masks to trigger the rapid release of purified dissolved oxygen, which boosts localized cell metabolism and aids skin detoxification. In hair care, CAT is used to limit chemical damage to the hair and scalp after bleaching with H_2O_2 by quickly neutralizing residual peroxide. However, catalase-based skincare formulations remain unstable because of environmental factors and chemical incompatibilities, and because of the difficulty of delivering and physically protecting this large, fragile enzyme against degradation, which also limits its penetration into skin or hair structures [45, 46].

5.2. Food Industry

In the food industry, H_2O_2 is widely used as disinfectant, particularly in dairy products. It is also added to milk to extend its shelf life [47]. In pasteurized milk and packaged beverages, in which H_2O_2 is widely used as an antimicrobial sterilizing agent, food regulations require that the final product be completely free of H_2O_2 before human consumption because of its toxicity (Table 2) [48]. In addition, the electrode can be adapted to other oxidases (such as alcohol oxidase, lactate oxidase, or amine oxidase) to measure ethanol, lactic acid, or biogenic amines as spoilage indicators in meat and fish over wide dynamic ranges [49].

5.3. Textile Industry

In the textile industry, CAT offers significant ecological, technical, and economic benefits over heavy chemical reducing agents, such as sodium thiosulfate, sodium hydrosulfite, or sodium bisulfite, and over multiple hot-water rinses, for removing residual peroxide after fabric bleaching and before dyeing (Table 2) [50]. In contrast to these agents, catalase specifically breaks down H_2O_2, which significantly decreases water consumption, and single-bath processing drastically reduces the total processing time, thermal energy consumption, and overall labor [51].

CAT also shows narrow substrate specificity for H_2O_2, which reduces the risk of accidental dye destruction or fiber damage. Moreover, it offers eco-friendly wastewater advantages over chemical agents, which increase the total dissolved solids and salts in the effluent, thereby helping processing facilities to meet strict environmental regulations [52].

5.4. Environmental Protection and Wastewater Treatment

In environmental monitoring, catalase is widely used as a rapid biomarker of environmental health. Measuring catalase activity in aquatic environments and correlating it with the biochemical oxygen level helps to evaluate microbial density, changes in organic loading, and overall metabolic health in rivers, stabilization ponds, and activated sludge systems [53]. Soil catalase activity is likewise a critical indicator of ecological health and functionality; however, external stressors, such as excessive nitrogen

deposition, can depress soil microbial diversity and, consequently, restrict catalase and other key ecological enzymes (Table 2) [54].

Catalase is also used in pollutant remediation, where it assists direct degradation and protects the primary microbial degraders from co-oxidation by complex environmental pollutants, such as phenols, pesticides, synthetic textile dyes, and polycyclic aromatic hydrocarbons. CAT scavenges the excess H_2O_2 produced in contaminated environments, shielding the host microbes and their metabolic pathways from oxidative damage and allowing remediation to continue [55, 56].

The applications of CAT in wastewater management span environmental monitoring, pollutant bioremediation, and agricultural development. The most suitable setting for sustainable wastewater management by CAT is semiconductor industrial wastewater, because semiconductor fabrication generates large volumes of H_2O_2 that must be treated before being recycled or safely discharged into subsequent biological treatment systems [57].

5.5. Agriculture and Plant Biotechnology

In plants, catalase is a cornerstone of the antioxidant defense system required to cope with environmental stressors, such as drought, high salinity, and heavy-metal contamination, which result in the overproduction of ROS. CAT mitigates ROS- induce damage to preserve cellular integrity and maintain the photosynthetic machinery (Table 2) [58].

5.6. Biosensors and Analytical Applications

In biosensor design, catalase is used as an auxiliary or reporter enzyme, depending on the biological framework and the transduction mechanism of the biosensor platform [59]. It is usually used as an auxiliary enzyme in oxidase-based conductometric biosensors for point-of-care monitoring of blood glucose. Compared with mono-enzyme glucose oxidase biosensors, this design offers advantages, especially at high glycemic levels, by preventing the accumulation of toxic H_2O_2 and converting it into oxygen that fuels the reaction (Table 2). This enhances signal amplification and the longevity of the biosensor and ensures highly repeatable and reliable diagnostic results during consecutive patient tests [47]. In bioremediation applications, catalase is frequently immobilized on support matrices to improve its stability against harsh environmental inhibitors, such as fluctuations in pH and temperature. It has also been integrated with paired fungal and bacterial strains to establish robust, cooperative degradation pathways [60].

5.7. Pharmaceutical and Medicinal Applications

5.7.1. Antimicrobial and Therapeutic Applications

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is classified as neither Gram-positive nor Gram-negative because of its thick cell wall, which contains mycolic acids (long-chain fatty acids) and waxes [4]. It is called an acid-fast bacillus because it resists the acid-alcohol decolorizer (a mixture of ethanol and hydrochloric acid) in the Ziehl-Neelsen (ZN) stain (acid-fast stain) [61]. The

bacterium is aerobic and produces catalase as a protective agent against ROS. Catalase, together with other proteins, enhances its intracellular survival in phagocytic cells [4, 24]. *M. tuberculosis* can grow intracellularly and is a slowly growing organism that divides every 12–24 hours and requires up to 8 weeks to develop visible colonies; caseous material is also present in the lesions [62]. Some active drugs cannot reach their target sites because of the effects of metabolism and their inability to penetrate the lesion, and the long division time may further decrease the peak drug level. These factors make the treatment of TB very difficult [63].

For example, cell wall inhibitors require actively dividing bacteria with continuous synthesis of cell wall components [64]. Isoniazid (isonicotinic acid hydrazide, INH) is an antitubercular cell wall inhibitor that affects the synthesis of mycolic acids [65]. It is a prodrug that is activated inside viable bacteria by the action of intracellular KatG (Table 1) [64]. The activated form of INH acts as a bactericidal agent by interfering with mycolic acid synthesis, leading to bacterial death. It is used in combination with three other drugs to prevent antibacterial resistance [4].

5.7.2. Pharmaceutical Manufacturing

Catalase is also used as an auxiliary enzyme coupled with a primary biocatalyst, such as amino acid oxidase or alcohol oxidase, to provide in situ protection of the primary enzyme against the corrosive by-product H_2O_2. This allows lower enzyme loading, reduces production costs, and improves the overall yield of active pharmaceutical intermediates, such as chiral alcohols and carbonyl compounds used in the synthesis of antiviral, cardiovascular, and anti-hyperlipidemic drugs [66, 67].

In green industries, catalase of bacterial origin is an eco-friendly, biodegradable alternative to harsh chemical reducing agents [68]. It is a highly efficient oxidoreductase that degrades toxic H_2O_2, a strong oxidizing agent used as a disinfectant in sterile filling lines and isolators for active pharmaceutical ingredients [69]. By breaking H_2O_2 down into harmless molecular oxygen and water, CAT protects reaction systems and biological molecules from oxidative degradation.

In addition, CAT supports aerobic bioprocesses by providing a steady source of oxygen in closed industrial bioreactors. This keeps the primary enzymes supplied with oxygen and drives the thermodynamic equilibrium of the cascade reaction toward the desired product without aggressive external or mechanical aeration, thereby protecting the enzymatic reaction from shearing and foaming [70, 71].

5.7.3. Microbial Diagnosis and Identification

A. Urinary Tract Infection Screening

The catalase test is a useful rapid screening tool for catalase-producing bacteria in urine specimens. An appropriate amount of urine is added to a tube, H_2O_2 is added, and the mixture is gently mixed. The formation of bubbles indicates a positive result [5].

B. Catalase-Based Biosensors for Microbial Diagnosis

CAT also serves as a powerful native reporter element for diagnosis and phenotypic fingerprinting in whole-cell biosensors. These biosensors exploit the endogenous enzymatic machinery of target pathogens by monitoring how bacterial cells decompose hydrogen peroxide, which allows immediate testing of wild-type clinical or environmental strains without genetic engineering [72]. Diagnostic platforms couple CAT with signal detectors, such as colorimetric or electrochemical sensors, for the fast detection of the presence, viability, and concentration of a target bacterium, because catalase has an exceptionally fast rate of substrate decomposition among all known enzymes [73, 74].

These advances guide patient care, for example, through the detection of *S. aureus*, a major catalase-positive pathogen found in wounds and systemic infections, with highly sensitive colorimetric biosensors [75], or through the detection of *Pseudomonas aeruginosa* in respiratory biofilms, using catalase and other metabolic markers to liquefy respiratory samples while simultaneously detecting the physiological activity of the underlying bacterial colony [76].

Table 2

Summary of the different applications of microbial catalases

Field	Application	Role and source
Cosmetic industry	Anti-aging formulations	It acts as antioxidant and protective against UV radiation and ROS [43, 44].
	Skin-care formulations	protect skin collagen and elastin structures. It acts as anti-inflammatory effect against skin irritation [44]
	Hair-care products	removes and neutralizing residual H_2O_2 following oxidative hair treatments [45, 46].
Food Industry	Monitoring of food quality and spoilage; removal of residual H_2O_2 from milk and beverages [47–49]	Decomposes H_2O_2 used as a sterilizing agent so that the final product is H_2O_2-free; amplifies the electrochemical signal. *Aspergillus niger* and *Micrococcus luteus* (microbial sources of food-grade catalase) [101, 102]
Textile Industry	Bleach termination.	Removal of residual H_2O_2 after fabric bleaching and before dyeing; treatment and reuse of bleaching effluent [50–52]. The enzyme is isolated from *Corynebacterium diphtheriae*, and *Burkholderia cepacia* [77].
Environmental Protection and Wastewater Treatment	Biomarker of environmental and soil health; bioremediation of pollutants (phenols, pesticides, dyes); treatment of H_2O_2-rich industrial wastewater [53–57]	Indicates microbial density and metabolic health; scavenges excess H_2O_2 and protects the degrading microbes from oxidative damage. Activated-sludge and soil microbial communities [53, 54]; paired fungal and bacterial strains [60].
Agriculture and Plant Biotechnology	Improvement of plant tolerance to drought, salinity, and heavy-metal stress [58]	Inoculation increases catalase activity in the host plant, which limits ROS accumulation and preserves cellular integrity [58]. Plant growth-promoting bacteria, e.g., *Pseudomonas frederiksbergensis*, *P. vancouverensis*, *Azospirillum brasilense*, *Azotobacter chroococcum*, and *Bacillus tequilensis* [78]
Biosensors and Analytical Applications	The auxiliary catalase prevents the accumulation of toxic H_2O_2.	auxiliary catalase can convert toxic H_2O_2 into oxygen that fuels the reaction [47]. This auxiliary catalase is isolated from *Aspergillus niger* [79]
Pharmaceutical and Medicinal Applications	Activation of the anti-tubercular prodrug isoniazid.	KatG of *M. tuberculosis* can intracellularly activate INH to become an active bactericidal agent and cell wall inhibitor, by interfering with mycolic acid synthesis [64, 65].
	Catalase is used as an auxiliary enzyme coupled with a primary biocatalyst to	It degrades toxic H_2O_2, a strong oxidizing agent used as a disinfectant in sterile filling

Field	Application	Role and source
	protect against the corrosive by-product H_2O_2.	lines and isolators for active pharmaceutical ingredients [69]

C. Microbial Identification

The catalase test is useful for the presumptive identification and differentiation of bacteria [80]. The principle of this test is based on the ability of bacteria to produce catalase, an enzyme that efficiently mediates the decomposition of H_2O_2 [24]. The enzyme is detected either by adding a drop of an appropriate concentration of freshly prepared, diluted H_2O_2 reagent to a small inoculum of bacterial colonies on a glass slide, or by adding a small inoculum of colonies to a few drops of the reagent in a test tube [5, 81]. The decomposition yields water and gaseous oxygen, which appear rapidly as bubbles after the colonies are exposed to the reagent [13, 82]. The slide method and the tube method are the two main methods used to perform the catalase test [13].

Although most obligate aerobic and facultative anaerobic Gram-positive and Gram-negative bacteria produce catalase, the test is used to differentiate catalase producers from catalase non-producers [83]. Performing the catalase test on colonies growing on media is not sufficient on its own, because it is only a presumptive test for identification and differentiation [84]; therefore, additional staining procedures and biochemical, serological, or molecular tests may be used to confirm the identification of species or strains [85].

The catalase test helps to differentiate catalase-positive *Staphylococcus* species from catalase-negative *Streptococcus* species. The test is performed after the presence of Gram-positive cocci has been established by microscopic examination of Gram-stained smears [80]. It is also helpful for differentiating *Listeria monocytogenes*, a short Gram-positive rod (coccobacillus), from *Streptococcus* species, because both produce similar narrow zones of β-hemolysis on blood agar [4, 86]. In addition, it helps to differentiate aerobic Gram-positive spore-forming bacilli of the genus *Bacillus* (catalase-positive) from aerotolerant strains of *Clostridium* species (catalase-negative) [87]. The test is also used to identify some microaerophiles, such as *Campylobacter* species, in order to distinguish catalase-positive *C. fetus*, *C. jejuni*, and *C. coli* from other catalase-negative *Campylobacter* species [80]. When the concentration of the H_2O_2 reagent is increased to 30%, the test can be used to differentiate *Neisseria gonorrhoeae*, which gives a strong positive reaction with vigorous bubbling, from other *Neisseria* species, which give only weak reactions [88].

Several precautions and limitations must be observed to ensure the accuracy of the catalase test by both the slide and tube methods.

Table 3
Summary of the different precautions and limitations for the accuracy of the catalase test

Precautions and Limitations	Explantation/ rational
Catalase testing should be performed in a microbiological safety cabinet	Some bacteria can be hazardous [89].
A 3% H_2O_2 solution should be used for routine catalase testing, while the 30% H_2O_2 reagent should be handled with appropriate precautions.	A 30% concentration is better for all catalase tests but is corrosive and causes severe skin damage, so 3% is commonly used for most bacteria [90]. If the skin is exposed to the concentrated reagent, wash the area with 70% ethyl alcohol rather than water [91].
The test should be performed under aseptic conditions using clean, dry glass slides or tubes.	Catalase-producing bacteria on the skin or in the air may contaminate the test [92]; dirt or suspended particles may cause false-positive results [93].
Hydrogen peroxide reagent should be stored in a dark-colored bottle at 2–8°C and freshly prepared before each test.	The reagent is very sensitive to high temperature and light and is unstable; exposure to sunlight decomposes it into water and oxygen, which reduces the efficacy of the test and gives false-negative results [80, 90].
Positive and negative control organisms should be included in daily quality control procedures before testing unknown isolates.	This ensures the efficacy of the reagent [94].
Colonies grown on blood agar should not be used for the catalase test.	The red blood cells (RBC) in this medium contain catalase or glutathione peroxidase, which degrade H_2O_2 and give false-positive results [83]. Use colonies from nutrient agar (non-fastidious bacteria) or chocolate agar (fastidious bacteria) [24, 95]; if only blood agar colonies are available, take the inoculum from the top of the colony [83].
Colonies grown on Mueller–Hinton agar should not be used for the catalase test.	The starch in this medium can interfere with H_2O_2 and produce bubbles that lead to false-positive results [95].
Fresh bacterial colonies from cultures incubated for 18–24 hours should be used and, bacterial cultures grown in liquid media should not be used for the slide catalase test.	Catalase is produced by live and active bacteria, so older colonies may give false-negative results [96]. Liquid media may contain several types of bacteria, which complicates the interpretation of the results.
The correct sequence of laboratory tests should be followed; for example, Gram staining should be performed before the catalase test.	If the catalase test is performed before Gram staining, a positive result does not indicate staphylococci, because most aerobic and facultative anaerobic Gram-negative bacilli are catalase-positive [96].
In the slide method, the H_2O_2 reagent should be added directly to the bacterial colonies	Adding the colonies to the reagent, shaking, or vigorous mixing releases dissolved O_2 as bubbles that can be mistaken for a true positive result [80, 83].

Precautions and Limitations	Explantation/ rational
without shaking or vigorous mixing.	
Metal loops (e.g., nichrome or iron wire) should not be used; platinum or plastic loops, or sterile wooden or plastic sticks, should be used instead.	Metal can react with H_2O_2 on contact and give false-positive results [97–99].
Weak catalase reactions should be interpreted with caution and, if necessary, confirmed using an appropriate alternative method.	Catalase-negative bacteria, e.g., *Enterococcus* species, may give weakly positive results because of pseudocatalase (non-haem) [24] or a peroxidase that slowly degrades H_2O_2 [5]; the presence of a few tiny bubbles after 30 seconds is considered negative [91]. Growth on haem-containing media may supply the haem necessary for enzyme production [93], and 1% glucose in the medium prevents pseudocatalase production by *Lactobacillus* species [100].
A negative slide catalase test should not be considered sufficient to exclude *S. aureus* infection.	Some strains give negative results; repeat the test by the tube method [98] or perform the α-aminolevulinic acid (ALA) test, which detects catalase-negative *S. aureus* [98, 99].
Anaerobic colonies should be exposed to atmospheric oxygen for 30 minutes before performing the catalase test.	This allows aerobic respiration [97].
Organisms other than those commonly mentioned may also produce positive catalase test results.	Fungi such as *Candida* and *Aspergillus* species can produce catalase [36]. All medically important genera of the *Enterobacteriaceae* are catalase-positive; the only strain unable to produce catalase is *Shigella. dysenteriae* type 1 [100].

6.Future Directions

Future research on microbial catalase should focus on developing species-specific catalase inhibitors without cross-reactivity with human catalases, providing a novel class of antimicrobials. The selective inhibition of microbial catalase may expose the target microbes to toxic reactive oxygen species resulting from the respiration process, thereby killing them. It also weakens microbial antioxidant and antiphagocytic defenses.

Research that focuses on improving microbial diagnosis using conventional catalase-based methods aims to increase the sensitivity and specificity of tests while integrating the catalase test into automated techniques.

7. Conclusions

Catalase is a key enzyme in the defense of aerobic and facultative anaerobic bacteria against oxidative stress. By decomposing H_2O_2 into water and oxygen, it supports bacterial survival, reduces the efficacy of H_2O_2-based antiseptics, protects pathogens against phagocytic oxidative killing, and gives catalase-

producing bacteria a competitive advantage over H_2O_2-producing species. Its stability, specificity, and high catalytic efficiency have made microbial catalase valuable in the biotechnological tool in the cosmetic, food, textile, pharmaceutical, agricultural, and environmental sectors, and as an auxiliary or reporter enzyme in biosensors for clinical, food, and environmental monitoring. In medicine, catalase-peroxidase activity is essential for the activation of isoniazid in *M. tuberculosis*, which illustrates the therapeutic relevance of bacterial catalases. In the laboratory, the catalase test remains a simple, rapid, and inexpensive presumptive test for the differentiation of bacteria, provided that its limitations are recognized and the recommended precautions are followed, including the correct reagent concentration, suitable culture media and inoculation tools, fresh cultures, appropriate controls, and the correct order of tests. Future work should focus on improving the stability and delivery of catalase in commercial formulations and on exploring bacterial catalases as targets for new antibacterial strategies.

Abbreviations

ALA
α-aminolevulinic acid
ATP
adenosine triphosphate
CAT
catalase
CO_2
carbon dioxide
H_2O
water
H_2O_2
hydrogen peroxide
INH
isoniazid (isonicotinic acid hydrazide)
Kat
catalase
KatE
catalase HPII
KatG
catalase-peroxidase
NADH
nicotinamide adenine dinucleotide (reduced form)
O_2
oxygen
RBC
red blood cell

ROS
reactive oxygen species
SOD
superoxide dismutase
TB
tuberculosis
UV
ultraviolet
ZN
Ziehl-Neelsen.

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Data availability

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Code availability

Not applicable.

Conflict of interest

The authors declare no conflicts of interest.

Author Contributions

ANMG, ISN, MAS and MAK contributed to the conceptualization of the review, development of the review objectives, literature search, data collection, and preparation of the original manuscript. HAAM and SMAK contributed to literature screening, data extraction, and critical analysis of the retrieved literature. BQMA, AA and MBM contributed to the interpretation and synthesis of the findings and preparation of tables and figures. ANMG, and ISN also supervised the review process and critically revised the manuscript. All authors read and approved the final manuscript.

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Figures

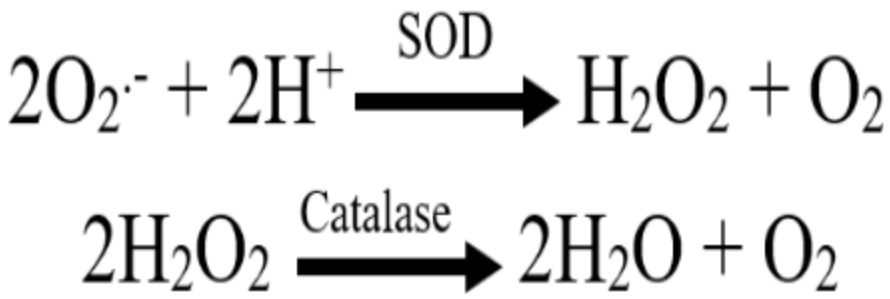


Figure 1

Enzymatic reactions involved in the decomposition of ROS [13].