

REVIEW



Genetic attributes of industrial enzymes of actinobacteria

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ABSTRACT

Actinomycetes represent a largely overlooked source of industrially significant enzymes and bioactive metabolites. Extensive studies ensure a comprehensive understanding of ecological diversity, regulatory networks, and metabolic optimisation related to enzyme synthesis. This review consolidates developments in isolation methods, metabolic regulation, and the biotechnological applications of actinomycetes. Pre-treatment method enables the isolation of actinobacteria. Furthermore, the regulatory pathways controlled by global switches like GlnR and DasR, which influence nitrogen metabolism and chitin utilisation, are inadequately characterised in numerous non-model species. The relationship between post-translational enzyme regulation, such as acetylation, and secretion processes through the Twin-Arginine Translocation (TAT) pathway requires detailed molecular analysis to improve extracellular enzyme production. Additionally, the application of bioinformatics-driven genome mining and synthetic biology methods remains limited in the prediction, expression, and engineering of multi-enzyme complexes with industrial applications. This review classifies actinomycetes as microbial dynamic biological systems, whose regulatory precision can be utilised for vital enzyme production.

KEY WORDS

Actinomycetes; Enzyme biosynthesis; Metabolic regulation; Global regulators (GlnR, DasR); Posttranslational modification; Twin-Arginine Translocation (TAT) pathway

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Introduction

Actinobacteria, a group of gram-positive, filamentous microorganisms, exhibit characteristics of both bacteria and fungi. The term Actinomycetes is derived from the Greek words "atkis" (ray) and "mykes" (fungus), reflecting their unique morphology and ecological roles [1]. These aerobic, spore-forming organisms are predominantly found in soil and aquatic environments, where they play a vital role in nutrient cycling and organic matter decomposition [2,3].

Actinobacteria are notable for their high guanine and cytosine content in DNA, which contributes to their genetic diversity and adaptability. They can be classified as chemoautotrophic or heterotrophic, with the majority being chemoheterotrophic, utilising a wide range of organic substrates, including polysaccharides. Their saprophytic nature allows them to thrive in various ecosystems, making them essential components of microbial communities [4].

The enzymatic capabilities of Actinobacteria have significant implications across various industries. In the dairy sector, microbial enzymes such as proteases and lipases enhance product quality and shelf-life. Similarly, in the baking industry, enzymes like amylases and xylanases improve bread texture and moisture retention. The beverage industry benefits from enzymes that facilitate juice processing, while the paper pulp industry utilises xylanases and cellulases to enhance deinking processes [5].

Actinobacteria also contribute to biotechnological advancements through the production of metabolic enzymes, including laccase, lipase, and amylase, which are pivotal in biofuel production from plant biomass [6]. Their ability to degrade complex organic substances, such as cellulose and hemicellulose, positions them as valuable agents in waste management and environmental remediation [7,3].

Actinobacterial enzyme research has expanded rapidly over the past two decades, necessitating a structured approach to literature selection for this review. Relevant publications were identified by searching major scientific databases, including PubMed, Scopus, Web of Science and Google Scholar, using combinations of keywords such as "Actinobacteria," "actinomycetes," "industrial enzymes," "cellulase," "lipase," "protease," "xylanase," "laccase," "chitinase," "keratinase," "fermentation," "regulation," and "twin-arginine translocation (TAT) pathway [1]." The primary search covered literature published between 2000 and 2025, with earlier seminal works included when necessary to provide historical or conceptual context.

Only studies that (i) clearly identified the actinobacterial strain to at least the genus level, (ii) provided methodological details for isolation, cultivation or enzyme production, and (iii) reported measurable outcomes such as enzyme activity, yield, stability, substrate range or application performance were considered for detailed discussion. Both laboratory-scale and pilot-scale investigations were included, whereas reports lacking experimental detail, patents without accessible data and purely descriptive notes were excluded. Additional references were obtained by screening the bibliographies of key articles and recent reviews. Although this review follows a narrative format, the use of explicit search terms and inclusion/exclusion criteria enhances the transparency, reproducibility and completeness of the literature synthesis.

Diversity and Habitat of Actinobacteria

Habitat

Actinobacteria, particularly the genus *Streptomyces*, predominantly inhabit terrestrial environments, with soil

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serving as their primary habitat. Numerous studies have consistently identified *Streptomyces* as the most abundant genus within this group, highlighting its ecological significance [7]. These terrestrial Actinobacteria are renowned for their antimicrobial properties, with several species capable of synthesising novel antibiotics that exhibit potent antibacterial activity.

In addition to arable lands, Actinobacteria such as *Micromonospora* and *Nocardia* have been identified in anoxic mangrove rhizospheres, albeit in lower densities due to tidal influences. Notably, *Nocardia* isolated from mangrove soils has been reported to produce cytotoxic metabolites that effectively inhibit human cell lines, including those associated with gastric adenocarcinoma [8]. Furthermore, extreme environments like desert soils support specific Actinobacterial species that utilize *Microcoleus* as a nutrient source. Research indicates the presence of Actinobacteria across various soil types, including sandy and alkaline soils, with *Streptomyces* being the dominant species, followed by *Nocardia*, *Nocardioopsis*, and other Actinomycetes [9].

Diversity

The importance of standardised and sterile techniques in soil sample collection to ensure the integrity of microbial analyses across diverse ecological environments. The geographical and environmental context of soil samples significantly influences the isolation outcomes. Elgawad et al. collected samples from local legume fields in Saudi Arabia, while Zhang et al. focused on subtropical regions in China, indicating that climatic factors such as temperature and humidity play a crucial role in microbial diversity. [10,11].

The collection of soil sample

Soil sampling is a critical aspect of ecological and microbiological research, as it provides insights into the diversity and functionality of microbial communities. Various studies have documented the methodologies employed in soil sample collection across different geographical and ecological contexts.

Bajracharya et al. conducted soil sampling in Nepal, collecting samples from depths of 5 to 10 cm in areas such as Narayanghat, Ramnagar, and Baseni, using tightly sealed polyethylene bags to prevent contamination [12]. Similarly, Fitri et al. collected soil samples from Indonesia at a depth of 10 cm, ensuring 50 m between sampling points, and utilised sterile polyethylene bags for transport [13]. Subhash et al. focused on the rhizosphere and non-rhizosphere zones, collecting samples from a depth of 0-15 cm to analyse microbial diversity [14].

In a broader context, Liu et al. collected soil samples from Nyingchi city, China, at depths ranging from 2 to 15 cm, while Sapkota et al. collected samples from organically cultivated fields and riverbanks in Nepal, emphasising the importance of sterile collection methods [15,16]. Chaudhary et al. documented soil sampling in Madhya Pradesh, India, from mining areas, highlighting the variability in soil characteristics across different habitats [17].

Ibnouf et al. further contributed to the understanding of soil diversity by collecting samples from various locations in Nepal and Indonesia, respectively. Additionally, Gurung et al. and Semwal et al. focused on specific depths of 4-5 cm and 3-5 cm, respectively, to assess microbial communities in local areas [18,19].

Research on desert soil has also been significant, with Bindushree et al. documenting soil sampling in the Taklamakan desert, noting extreme temperature variations and minimal

rainfall [20]. Researchers further expanded this research to include various deserts across Asia, emphasising the need for careful sampling techniques in harsh environments.

Rhizosphere soil sampling has been explored by Hellmuth and Brink in 2013, who collected samples from plant root zones to study the associated microbial communities [21]. Marine soil samples were collected by Gobalakrishnan et al, focusing on sediment analysis from coastal regions, which further illustrates the diversity of methodologies employed in soil sampling [22].

Collectively, these studies demonstrate that actinobacterial diversity is strongly shaped by edaphic factors, vegetation type, and climatic regime; however, similar genera, particularly *Streptomyces*, *Nocardia*, *Micromonospora*, and related taxa, recur across geographically distant sites. This recurrent pattern suggests that a relatively conserved core of ecologically versatile genera underpins enzyme-driven nutrient cycling in soils ranging from mangroves and agricultural fields to deserts and mining areas. At the same time, methodological differences in sampling depth, pre-treatment, and selective media formulation lead to pronounced variation in the taxa recovered, complicating direct comparison of diversity metrics between studies. Future work would benefit from harmonised sampling protocols and standardised isolation strategies that enable more robust cross-study comparisons and more accurate linkage between specific habitats and their dominant enzymatic profiles [11].

Isolation of Enzyme-Producing Actinobacteria

Pretreatment for selective isolation

Pre-treatment of soil samples, such as air drying or heat treatment, has been shown to enhance the isolation of actinobacteria by reducing competing microbial populations [19]. This step is critical for obtaining pure cultures and ensuring the dominance of actinobacteria in the isolated samples. The isolation of actinobacteria, particularly from soil samples, necessitates pretreatment strategies to mitigate contamination and enhance enzyme production and activity. Various pretreatment techniques, including physical and chemical methods, have been developed to optimise the isolation process [23,24].

Comparison of isolation strategies across studies reveals a clear trade-off between selectivity for rare or slow-growing actinobacteria and the overall cultivable diversity obtained. Aggressive physical or chemical pre-treatments, such as high-temperature incubation or phenol treatment, efficiently suppress fast-growing bacteria and fungi but may simultaneously eliminate sensitive actinobacterial taxa with valuable enzymatic traits. In contrast, milder treatments combined with broad-spectrum media (e.g. TSA, YMA) tend to recover a wider taxonomic range but with reduced enrichment for high-performing enzyme producers. Likewise, reliance on a limited set of media, such as starch casein agar or actinomycete isolation agar, biases the community towards strains adapted to those substrates. These methodological differences help to explain discrepancies in reported enzyme-producing frequencies among studies and highlight the need for multi-media, multi-pretreatment approaches when the goal is to prospect for novel or industrially robust enzyme producers.

Physical pretreatment methods

Physical pretreatment methods are pivotal in isolating

actinomycetes by promoting their growth while inhibiting unwanted Gram-negative bacteria. Common techniques include air drying, hot-air treatment, moist incubation with radiation, and centrifugation. Actinomycete spores demonstrate significant resistance to desiccation, which allows for selective growth under controlled conditions. For instance, heating soil samples at 120°C for one hour has been shown to favour the growth of *Streptomyces* and other rare genera, such as *Spirilliplanes* and *Actinomadura*, on humic acid vitamin (HV) agar [24]. Furthermore, pre-incubation at 110°C for ten minutes effectively suppresses the growth of competing bacteria and fungi. Notably, Yamamura et al. introduced a sucrose gradient centrifugation method specifically for isolating *Nocardia* species, highlighting the diversity of physical techniques available [24].

Chemical pretreatment methods

Chemical pretreatment methods also play a crucial role in the selective isolation of actinomycetes. Various agents, including calcium carbonate, chitin, and phenol, serve as carbon and nitrogen sources, thereby supporting actinobacterial growth. For example, treating air-dried soil with calcium carbonate and incubating it at 28°C has been shown to significantly enhance actinomycete colony counts compared to untreated samples [25]. Specific chemical treatments can selectively isolate genera such as *Streptomyces*, *Micromonospora*, and *Actinomadura*. For instance, the application of 0.05% sodium dodecyl sulfate (SDS) and 5% yeast extract has been effective in promoting the growth of *Streptomyces* on nalidixic acid-containing HV agar.

Actinobacteria, a group of Gram-positive bacteria, are known for their significant role in soil ecology and their potential in biotechnological applications, particularly in enzyme production. Various methodologies have been employed to isolate these microorganisms from soil samples, each with distinct protocols and media compositions.

Isolation

Serial dilution method

A common approach involves serial dilution of soil samples in sterile distilled water, followed by inoculation onto selective media. For instance, Bajracharya et al. described a method where 1 g of soil is diluted in 10 ml of distilled water, and subsequent dilutions (10² to 10⁻³) are plated on starch M-protein agar for incubation at 28°C [12]. Similarly, Fitri et al. utilised Yeast Malt Agar (YMA) for isolation, emphasising the importance of maintaining specific incubation temperatures to promote growth [13].

Selective media applications

Various media have been employed for the isolation of actinobacteria, including Starch Casein Agar (SCA), Tryptic Soy Agar (TSA), and Actinomycete Isolation Agar (AIA). For example, Subhash et al. isolated actinobacteria on skimmed milk agar and tyrosine agar, while Liu et al. reported successful isolation using TSA and TSB, highlighting the versatility of media in promoting actinobacterial growth [14,15].

Preliminary Characterisation of Isolates

Actinobacteria, a phylum of Gram-positive bacteria, are distinguished by their diverse morphological forms and notable enzymatic capabilities. These bacteria predominantly exhibit rod-shaped or filamentous forms, creating complex networks like fungal mycelia. Many genera produce true branching hyphae, which can be straight, twisted, or septate, and differentiate into substrate and aerial mycelium [25]. The substrate mycelium arises from germinating spores and displays various branching patterns, while aerial mycelium, which plays a role in reproduction, is characterised by a fibrous sheath [26]. A key reproductive feature of many Actinobacteria is asexual reproduction through spore

formation, often accompanied by mycelial fragmentation, contributing to their morphological diversity.

After isolation, actinobacteria are characterised based on morphological and physiological traits. Techniques such as Gram staining, spore chain structure analysis, and biochemical tests are commonly employed [27,28]. The screening for enzyme production, particularly amylase, cellulase, and lipase, is a focal point in many studies. For instance, Bindushree et al. utilised the starch agar plate method to assess amylase activity, while focused on lipase production using Tween-80-supplemented media [20].

Taxonomic classification at the genus and species levels primarily relies on microscopic morphology and chemotaxonomy, which encompasses the analysis of cell wall composition and whole-cell sugar distribution [17].

Enzymatic Properties of Actinobacteria

Actinomycetes produce enzymes via a meticulously organised biological assembly line, governed by three essential steps:

Fuel and building blocks

The fundamental metabolism of the cell functions as a driving force, converting simple sugars into essential precursor building blocks (such as amino acids and fatty acids) and energy cofactors required for the synthesis of large enzyme molecules.

The regulatory switch

Global regulators, including GlnR and DasR, act as key switches, interpreting environmental signals such as the availability of nitrogen or chitin. The cell's metabolic priority is determined by the decision to either allocate resources for the synthesis of new enzymes (proteins) or to activate catabolic enzymes (such as amylase or chitinase) for the breakdown of external nutrients [15].

Assembly and export

Following synthesis, the immediate activity of these enzymes is intricately regulated through chemical modifications, such as acetylation, which serve as a rapid response mechanism. Ultimately, extracellular enzymes intended for industrial use are exported from the cell in their fully folded conformation through the specialised TAT pathway [29].

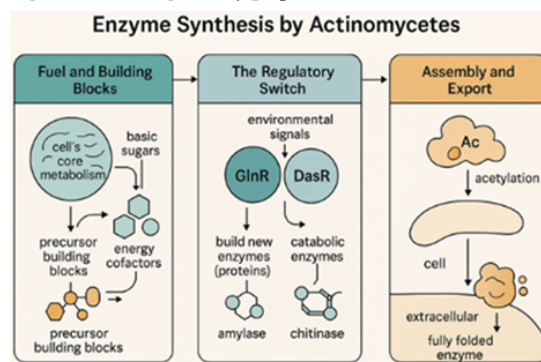


Figure 1. Schematic representation of the regulatory architecture governing enzyme synthesis in Actinobacteria. Central carbon and nitrogen metabolism generate the precursor molecules and energy required for enzyme biosynthesis, while global regulators such as GlnR and DasR integrate environmental signals (e.g. nitrogen and chitin availability) to modulate transcription of catabolic and biosynthetic genes. Post translational modifications, including acetylation, provide rapid fine-tuning of enzyme activity in response to changing conditions. Fully folded extracellular enzymes are subsequently exported via the TAT pathway, enabling the degradation of complex substrates in the surrounding environment.

When considered collectively, the current data point to a scenario in which tightly integrated regulatory hierarchies that link primary metabolism to environmental sensing and secretory capacity regulate the synthesis of actinobacterial enzymes. By coordinating nitrogen and carbon flux with the activation of extracellular hydrolases and oxidoreductases, global regulators like GlnR and DasR do more than just alter individual pathways; they also align enzyme synthesis with the availability of nutrients. The TAT route guarantees that completely folded proteins reach the extracellular space, whereas post-translational modifications increase this control by quickly varying enzyme activity in response to changing situations. But most mechanistic insights come from a limited number of model strains, which means that the regulation logic of extremophilic and non-model actinobacteria is mostly unknown. To logically create strains suited to certain industrial processes, regulatory research must be expanded to include these understudied species.

Industrially Important Enzymes

Actinobacteria, particularly the genus *Streptomyces*, are recognised for their ability to produce a diverse array of industrially significant enzymes. This review focuses on several key enzymes, including cellulases, lipases, proteases, pectinases, keratinases, chitinases, xylanases, and laccases, highlighting their production, applications, and potential for industrial use.

Cellulases are critical for hydrolysing cellulose, a complex carbohydrate, by breaking down glucosidic bonds. *Streptomyces* species, such as *S. drozdowiczii*, *S. lividans*, and *S. rutgersensis*, are notable cellulase producers utilised in industries ranging from pulp and paper to biorefineries. The cellulolytic capabilities of *Streptomyces viridobrunneus* demonstrate the potential for converting agro-industrial waste into valuable products. Cellulases find applications in juice colour extraction, detergent formulation, and biomass pretreatment [30].

Lipases, including cholesterol esterases, are vital for hydrolysing lipids and have applications in clinical diagnostics and organic synthesis. *S. lavendulae* and *Rhodococcus erythropolis* have been identified as sources of lipases with varying substrate specificities and optimal activity conditions [31]. The growing interest in lipases with high stereospecificity underscores their potential in resolving racemic mixtures.

Proteases, which catalyse the cleavage of peptide bonds, are among the largest classes of industrial enzymes, accounting for approximately 60% of global enzyme sales [32]. *Streptomyces* strains are significant producers of alkaline proteases, which are utilised in textiles, leather, and the food industries. Recent studies have highlighted the potential of proteases in waste management and cancer treatment, showcasing their versatility in various applications.

Pectinases catalyse the hydrolysis of pectic polymers and are crucial in the food industry for improving juice clarity and flavour [33]. Their ability to reduce turbidity in fruit juices makes them valuable in juice production processes.

Keratinases, produced by *Streptomyces* and *Actinomadura*, are essential for recycling keratin-rich waste materials, such as feathers and hair. This biotechnological approach offers a sustainable solution for waste management [34].

Chitinases play a significant role in biocontrol by hydrolysing chitin, a component of fungal cell walls. Their application in

agriculture for enhancing plant resistance to pests and diseases is promising, as is their potential for managing seafood waste [35].

Xylanases, primarily produced by *Streptomyces*, target xylan in hemicelluloses, making them valuable in the pulp and bio-bleaching industries. Their thermostability enhances their utility in various industrial applications.

Several cross-cutting trends emerge across enzyme classes. *Streptomyces* species continue to be the primary industrial sources of cellulases, proteases, xylanases, and laccases, due to their genetic tractability and proven fermentation capabilities. However, there is a growing body of evidence highlighting the potential of less-studied genera found in extreme or specialised environments. Many actinobacterial enzymes demonstrate advantageous properties, including thermostability, functionality at alkaline pH, and resistance to detergents or organic solvents, which are well-suited to contemporary industrial requirements. Discrepancies in assay conditions, substrates, and reporting units often hinder quantitative comparisons of activities across studies. In conclusion, although numerous enzymes have progressed from proof-of-concept studies to pilot-scale testing, there remains a lack of systematic optimisation regarding production titres, downstream processing costs, and long-term operational stability. Addressing these issues is essential for converting the extensive enzymatic capabilities of Actinobacteria into economically viable biocatalysts.

Production and Confirmation of Actinobacterial Enzyme

The enzymatic capabilities of actinomycetes are popularized and it is significant due to their diverse applications in biotechnology and industry. A study by Bajracharya et al. evaluated 31 actinomycete isolates from the Chitwan District, revealing that 83% produced gelatinase, 48% amylase, 41% cellulase, 45% lecithinase, and 61% urease [12]. This highlights the substantial enzymatic potential of these microorganisms, suggesting their utility in various fields.

Actinobacteria inhabit diverse ecological niches and are prolific producers of secondary metabolites with antimicrobial, anti-tumour, and antiviral properties [36]. They also yield a variety of industrially relevant enzymes, including lignin peroxidase, laccase, and tyrosinase, which are advantageous for sustainable and eco-friendly industrial applications.

Amylase production was assessed in selected bacterial isolates cultured in starch broth, demonstrating the enzyme's activity through the formation of a transparent halo around colonies on starch agar [30]. Similarly, xylanase production was evaluated using Birchwood xylan liquid medium, with activity confirmed through the dinitrosalicylic acid (DNS) method [37].

Lipase production was investigated using ISP2 medium, where clear zones around colonies indicated successful enzyme synthesis. In contrast, cellulase production remains economically challenging, primarily due to the high costs associated with submerged fermentation (SmF) technologies. Alternative methods, such as solid substrate fermentation (SSF), are being explored to enhance yield and reduce costs [20].

Chitinases, predominantly produced by actinobacteria, play a crucial role in breaking down chitin, with studies indicating significant chitinolytic activity among various isolates [20]. Pectinase production was confirmed through the appearance of halos on pectinolytic media, while catalase activity was assessed

by observing bubble formation upon hydrogen peroxide application [30].

Despite the variety of screening assays and production media used, it is consistently observed that a significant proportion of environmental actinobacterial isolates exhibit multiple enzyme activities concurrently, indicating an intrinsic ability for polysubstrate degradation. Most studies, however, are limited to qualitative or semi-quantitative plate assays, with only a few advancing to comprehensive kinetic characterisation, stability profiling, or process-level optimisation. The disconnect between initial screening and in-depth evaluation leads to a fragmented understanding of the strains and enzyme systems that are genuinely suitable for scale-up. Systematic workflows that integrate broad, high-throughput primary screening with targeted characterisation of the most promising candidates would significantly improve the predictive value of isolation studies and expedite the identification of enzymes with true industrial potential.

Bio-fermentation

Fermentation is a pivotal biotechnological process that facilitates enzyme activity by fostering microbial production and establishing an optimal environment for enzymatic functions. Microorganisms, including Actinobacteria, bacteria, yeast, and fungi, utilise fermentation to secrete enzymes that catalyse the degradation of complex substrates such as sugars, proteins, and fats into simpler compounds [32]. The fermentation process can be categorised into two primary methods: Solid State Fermentation (SSF) and Submerged Fermentation (SmF), each with distinct advantages and applications.

Solid State Fermentation (SSF)

Solid State Fermentation involves cultivating microorganisms on solid substrates with low moisture content, such as agro-industrial by-products like rice bran, sugarcane bagasse, and wheat bran. The choice of substrate is influenced by factors such as cost, availability, and moisture levels. SSF is particularly advantageous due to its higher product yields, reduced effluent production, and lower energy requirements compared to SmF. Additionally, SSF simplifies the fermentation process by eliminating the need for nutrient solubilization and facilitating easier recovery of end products [32]. The preferred microorganisms for SSF are typical Actinobacteria, which thrive in low-water environments.

Submerged Fermentation (SmF)

Submerged Fermentation, on the other hand, is characterised by the cultivation of microorganisms in a liquid medium containing dissolved nutrients, often in controlled environments such as bioreactors. This method allows for stringent monitoring of fermentation parameters, including pH and temperature, which are critical for optimising enzyme production. SmF is favoured in industrial settings due to its higher yields, reduced labour costs, and lower risk of contamination. For instance, *Bacillus subtilis* has been effectively utilised for protease production in SmF using agro-wastes as substrates [32].

Overall, both SSF and SmF present viable options for microbial enzyme production, each with unique benefits that cater to specific industrial needs. The ongoing refinement of these fermentation techniques, alongside advancements in machinery and processes, establishes their growing significance in biotechnological applications and food processing. [32]. Studies

on solid-state and submerged fermentation, when analysed collectively, reveal that there is no universally superior method for actinobacterial enzyme production; instead, the optimal configuration is specific to both the enzyme and the process involved. SSF typically yields greater volumetric productivity and minimises downstream processing for enzymes designed for direct application to solid substrates. In contrast, SmF allows for enhanced control over pH, aeration, and nutrient gradients, which are essential for the precise regulation of enzyme synthesis. Many comparative evaluations of solid-state fermentation (SSF) and submerged fermentation (SmF) are limited by short cultivation durations, suboptimal inoculum preparation, or the application of laboratory media that do not accurately represent industrial feedstocks. Future process development should integrate strain-level regulatory insights with techno-economic analyses to align each enzyme-substrate pair with the most appropriate fermentation platform.

Application of Enzymes of Actinobacterial Origin

The application of enzymes derived from Actinobacteria has gained significant attention across various industries due to their diverse functionalities and environmental benefits. This review explores the roles of these enzymes in feed, detergents, pharmaceuticals, leather, textiles, agriculture, probiotics, bioherbicides, pigments, and wastewater treatment.

In the feed industry, enzymes such as phytases, proteases, and xylanases enhance nutrient digestibility and feed efficiency, with the global feed enzyme market projected to reach \$730 million by 2015 [38]. Research continues to focus on developing heat-stable enzymes and novel polysaccharide-degrading enzymes to improve feed utilisation.

The detergent industry, accounting for nearly 60% of global enzyme production, utilises enzymes like proteases and amylases to enhance cleaning efficacy. Proteases derived from alkaliphilic *Bacillus* strains are particularly effective in laundry applications [32].

In pharmaceuticals, enzymes are crucial for treating enzymatic dysfunction and developing drugs for various diseases. Microbial enzymes such as glutaric acid acylase and D-amino acid oxidase are employed in synthesising pharmaceuticals, including biosynthetic human insulin [32].

The leather industry benefits from biodegradable enzymes that improve processing while reducing environmental impact. Enzymatic dehairing using proteases and lipases minimises the use of harmful chemicals, enhancing leather quality and reducing effluent toxicity [24].

In textiles, microbial enzymes facilitate sustainable production by replacing harsh chemicals in processes such as desizing and bio-finishing. Enzymes like cellulases and lipases are preferred for their efficiency and environmental safety [39].

Agriculturally, enzymes play a vital role in soil health and nutrient cycling. Research indicates that endophytes from mangrove plants exhibit significant enzymatic activity, enhancing soil fertility and pest resistance [32].

Probiotics derived from marine Actinobacteria show promise in aquaculture, enhancing growth and disease resistance in aquatic species [7]. Additionally, Actinobacteria produce bioherbicides that effectively control weeds, offering an eco-friendly alternative to synthetic herbicides.

Finally, enzymes are pivotal in wastewater treatment, degrading toxic pollutants and facilitating the bioconversion of hazardous

compounds into safer products. Enzymes such as cellulases and lipases are employed to treat industrial effluents, promoting sustainable waste management practices [5].

The extensive application of actinobacterial enzymes in various sectors, including feed, detergent, pharmaceuticals, leather, textiles, agriculture, and the environment, highlights their versatility while revealing several ongoing challenges. Enzyme formulations are often required to operate under extreme conditions, including high salinity, extreme pH, elevated temperatures, and the presence of surfactants and oxidants. Only a limited number of currently characterised enzymes can withstand these conditions. Furthermore, large-scale implementation is frequently limited by production costs, the necessity for cofactor regeneration, and the incorporation of enzymatic processes into current industrial workflows. Regulatory factors and consumer acceptance significantly influence the rate of adoption, especially in the domains of food and pharmaceuticals. Overcoming these limitations necessitates classical strain improvement and process optimisation, alongside the strategic implementation of genome mining, protein engineering, and synthetic biology to customise actinobacterial enzymes for targeted, high-value industrial applications.

Research Gap and Future Scope

Despite notable progress in the cataloguing of actinobacterial enzymes and their applications, several critical knowledge gaps persist. The predominant focus of functional studies is on a limited range of genera, chiefly *Streptomyces*, resulting in the underutilization of the enzymatic potential of numerous rare or uncultured actinobacteria. Secondly, the integration of ecological data, including habitat physicochemistry and community structure, with enzymatic phenotypes is limited, which obstructs the prediction of locations where novel biocatalysts are likely to be discovered. Third, the regulatory pathways connecting global nutrient sensing, stress responses, and secretion systems have been analysed in only a limited number of model strains, thereby constraining the rational design of high-performing production hosts.

Future research must focus on systematic bioprospecting of under-explored habitats through standardised sampling and isolation protocols, alongside culture-independent methods like metagenomics and single-cell genomics. The integration of genome mining, comparative genomics, and high-throughput expression platforms can expedite the discovery and characterisation of novel enzyme families and isoforms. Integrating systems biology-based modelling with bioprocess engineering is crucial for translating laboratory-scale findings into robust, scalable processes that achieve industrial performance and sustainability objectives.

Conclusion

Actinobacteria serve as a highly productive source of industrially significant enzymes, including hydrolases like amylases, cellulases, proteases, lipases, chitinases, pectinases, and xylanases, along with oxidoreductases such as laccases and peroxidases. These enzymes facilitate the deconstruction of structurally complex biopolymers and the transformation of various substrates, supporting a wide range of applications in food processing, textiles, detergents, pharmaceuticals, agriculture, and environmental bioremediation. The reviewed literature emphasises the diversity of actinobacterial enzymatic

repertoires and the critical influence of habitat diversity, isolation strategies, and regulatory networks on enzyme production profiles.

The analysis identifies significant limitations that currently hinder industrial deployment. Enzyme yields are often sub-optimal for numerous promising strains, and variability in assay conditions and reporting metrics hinders meaningful comparisons of performance across studies. The mechanistic understanding of global regulatory circuits and secretion pathways remains predominantly confined to a limited number of model organisms, thereby constraining the rational engineering of non-model taxa that are of industrial interest [37]. Moreover, economic and operational barriers, such as fermentation costs, downstream processing complexity, and process integration, often hinder scale-up efforts.

To address these challenges, a concerted shift is necessary from purely descriptive screening to integrate hypothesis-driven research that connects ecology, genomics, regulation, and process engineering. Expanding the taxonomic range of actinobacteria under investigation, utilising genome mining and synthetic biology for enzyme discovery and optimisation and incorporating techno-economic and life-cycle assessments into process development pipelines will be essential. These strategies enable the effective realisation of Actinobacteria's potential as dynamic microbial platforms for sustainable enzyme production, thereby facilitating the transition to greener, bio-based industrial technologies.

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