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Review article

MARINE ACTINOMYCETES AND THEIR BIOTECHNOLOGICAL APPLICATIONS – A REVIEW

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ABSTRACT

The unicellular filamentous bacteria known as the actinomycetes generate spores and build a branching network of filaments. They have long been acknowledged as the main causes of the unpleasant earthy-musty tastes and odours in water. According to a recent study that used both culture-dependent and culture-independent approaches, indigenous marine actinomycetes exist in the oceans and are widely distributed in a variety of marine environments. Marine actinomycetes are incredibly diverse and unusual organisms that live in aquatic environments. Novel actinomycetes have been isolated from various marine environments and ecosystems. These marine actinomycetes produce a variety of secondary metabolites. Many of these metabolites have biological functions and may one day be used as medicinal treatments. Marine actinomycetes are a rich source of previously untapped secondary metabolites. In this review, the classification, occurrence and habitat, enzymes present in actinomycetes and their applications have been discussed.

Keywords: actinomycetes, enzymes, antibiotics, guanine-cytosine content.

AIMS AND BACKGROUND

Actinomycetes are Gram-positive bacteria that resemble fungi and have a high-GC (Guanine-Cytosine content). They consist of a large number of secondary metabolites with diverse biological activities. Gram-positive bacteria are classified into two phylogenetic groups: “low-GC” and “high-GC”. GC content is the percentage of GC base pairs in an organism’s DNA (Deoxyribonucleic acid). Low GC individuals have more AT (Adenine-Thymine) base pairs in their DNA than high GC individuals.

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GC content, while a crude measure of microbe relatedness, is effective for distinguishing broad phylogenetic divisions. They have a wide range of life cycles that distinguishes them from other prokaryotes.

Gram-positive bacteria of the *Actinobacteria phylum*, Class Actinobacteria, Subclass Actinobacteridae, Order Actinomycetales, with ten suborders, over 30 families, and over 160 genera. Actinomycetes are a diverse group of microbial resources with a wide range of practical applications and a high commercial value. They generate a wide range of non-antibiotic bioactive metabolites, including enzymes, enzyme inhibitors, immunological regulators, anti-oxidation reagents, and so on. Actinomycetes are found in a wide range of natural environments, including soil and the sea[1].

There are millions of microorganisms that exist in the ocean, and they are crucial for the mineralisation of complex organic matter, the decay of dead plankton, plants, and animals, the breakdown of pollutants and toxicants, and the degradation of primary and secondary productivity. One of the most significant subgroups of marine microorganisms is the actinomycetes. They have a wide range of potentials for creating advantageous secondary metabolites.

Research in India with the goal of studying both mycological and bacteriological features, spanning near offshore and deep-sea waters has made significant progress in understanding the microbes of the seas around India. India is surrounded by seas on three sides and has a 7500-km-long coastline. This indicates a huge possibility for resources to be exploited. India utilised the new ocean regime to create an exclusive economic zone (EEZ) spanning approximately 2.01 million km^2, primarily dedicated to studying marine microorganisms. Because of a lack of suitable facilities, educated people, and adequate financial backing, their activity was limited to coastal areas.

Although people first focused on studies involving microbial engagement in biogeochemical cycles, they gradually expanded their scope of the investigation. Understanding the role of bacteria in phosphate utilisation, nitrogen fixation, and silicate solubilisation has taken a lot of research. During this time, several of the extreme halophiles were also studied, but the focus was on their involvement in salt pans and the deterioration of dried fish.

During this time period, researchers were also interested in the production of beneficial chemicals by marine microorganisms. The marine Streptomyces were the main focus of attention[2].

Various maritime sediments, such as mangroves, marine molluscs, and detritus, among others, were used to extract *Streptomyces* species. One of the most intriguing results was that only 20–25% of *Streptomyces* sp. cultures recovered from sediments were antagonistic to the test microorganism, compared to about 70% of *Streptomyces* sp. obtained from marine molluscs. Off the east coast at the time, a particular marine Vibrio sp. was found in marine sediments and it produced an antileukemic toxin (L-asparginase). When it came to the treatment of cancer at the time, this was demonstrated to be superior to commercial remedies[3].

CLASSIFICATION OF ACTINOMYCETES

The classification of actinomycetes based on their characteristics is tabulated in Table 1.

Table 1. Classification of actinomycetes

Section	Characteristics
Nocardioform actinomycetes	Aerobic, potentially acid-alcohol-fast, occurring as rods, cocci, and branched filaments or as substrate and aerial mycelium that fragments, wall chemotype IV, and containing mycolic acids
Dermatophilus	Atmospheric requirement is aerobic or capnophillic; metabolism is weekly fermentative
Actinoplanetes	Aerobic sporoactinomycetes, nonmotile, spores may be contained within vesicles; no aerial mycelium; wall chemotype II; whole-organism hydrolysates contain arabinose and xylose
Trueperella	Capnophilic atmospheric demand and fermentative metabolism
Streptomycetes and related genera	Aerobic sporoactinomycetes; form an extensively branched substrate and aerial mycelium
Thermoactinomycetes	Atmospheric requirement is aerobic. There are no thermophilic species. Meso-DAP is present in the cell wall, but no distinctive amino acids or sugars are present
Other genera	They all produce spore chains with the capacity for aerial development

Studies on microbiological pollution of coastal areas acquired attention throughout the first half of the second phase due to their significance in coastal zone management (1976–1985). A number of papers on the distribution of indicator bacteria and human disease in water, sediment, plankton, and faunal samples are becoming available throughout the Indian coast. Three new Salmonella serotypes, *S. irumu*, *S. panama*, and *S. lexington*, were found in environmental samples taken from the Pichavaram mangroves and velar estuary.

The halophilic *Vibrio parahaemolyticus* is another important bacterium in the marine environment that has attracted a lot of attention. This bacterium is the cause of gastroenteritis caused by seafood. A two-year rigorous and systematic assessment at Parangipettai revealed the presence of *Vibrio parahaemolyticus* in water, sediment, vegetation, and wildlife. In the last decade (1986–1995), research was conducted in a variety of areas and on a variety of creatures[4].

OCCURRENCE AND HABITATS OF ACTINOMYCETES

1. *Soil*. In most soils, actinomycetes make up a considerable portion of the microbial population. Actinomycetes counts of over one million per gramme are frequent, according to estimates. Soil has been used to isolate over twenty genera. Streptomycetes are common and have been reported to make up 95% of more than 5000 soil isolates[5]. The kind and population of actinomycetes in soil are influenced by environmental

conditions. In culture, most actinomycete isolates behave like neutrophils, with a pH range of 5.0 to 9.0 and an optimal pH of around 7.0.

The distribution and activity of soil actinomycetes are influenced by several environmental factors, including pH. Acidophilic and aciduric streptomyces are abundant in acidic soils below pH 5.0, whereas neutrophilic is rare. However, there are just a few reports of to 9.5 being isolated from soil near a Salt Lake. In the laboratory, most actinomycetes behave as mesophiles, with optimum growth temperatures of 25 to 30°C. In compost, many mesophilic actinomycetes are active. Still the ability of obligatory or facultative thermophilic actinomycetes to grow at temperatures above 40°C to self-heat during decomposition typically provides perfect conditions. Actinomycetes are vital in the decomposition of plants and other materials, particularly in the destruction of complex and refractory polymers.

Lignin, cellulose, and lignocellulose are all degraded by them. Actinomycetes have been found to degrade a variety of other naturally occurring polymers in soil, including hemicellulose, pectin, keratin, chitin, and fungal cell wall components. Actinomycetes derived from the rhizosphere are capable of impeding the growth of pathogens, while isolates from the rhizosphere can hydrolyse starch. Moreover, various organisms isolated from the rhizosphere exhibit the ability to produce gibberellin-like compounds. In culture and when inoculated into soil, several actinomycetes create indole acetic acid. Actinomycetes, notably Streptomyces, are important players in soil antagonistic interactions. Streptomyces from the rhizosphere have long been used to combat fungal root infections. Other than antibiotics, Streptomyces and fungi can have a variety of antagonistic interactions. An increase in the population of Streptomyces in the rhizosphere, which suppresses the pathogen by producing antifungal drugs, has been linked to a lower incidence of root infection. Plant diseases can be controlled biologically using actinoplanes[6].

2. *Compost and related materials*. In the early phases of decomposition, several mesophilic actinomycetes are active in compost. The ability to self-heat during decomposition, on the other hand, creates excellent conditions for obligatory or facultative thermophilic actinomycetes. Thermoactinomycetes and Saccharomonospora, for example, are strictly thermophilic genera. On animal manure, thermophilic actinomycetes thrive. They have worked with pig faeces fermentation, straw, and pig faeces deodorisation.

Streptomyces diastaticus and *Thermoactinomyces vulgaris* prevail in the spent, steamed compost and its dust, whereas Thermomonospora species thrive during the second indoor phase of manure preparation for mushroom growing. *Thermomonospora curvata* has been found to be involved in the degradation of municipal waste compost and the production of thermostable C1 (total cellulose) and Cx (exocellular endoglucanase) cellulose. Actinoplanes and related creatures can develop on plant litter in rivers and are prevalent in soils, rivers, and lakes. Micromonospora were thought to be native to freshwater ecosystems, and they had a role in cellulose, chitin, and

lignin turnover. Micromonospora was found in streams, rivers, and lake sediments by several researchers. *Streptomyces* spores are also washed into freshwater and marine environments on a regular basis. The presence of aquatic streptomyces and growth on the chitinous exoskeletons of *Procambarus versutus* immersed in a woodland stream has been asserted by certain researchers.

Actinomycetes alter the flavour and odour of drinking water, making it unappealing. Compounds generated by Streptomyces, such as geosmin and methyl iso-borneol, give the food an earthy flavour and odour. Actinomycetes and geosmin production can be aided by plant and animal waste at the water's edge[7].

3. *Marine habitats*. Actinomycetes were mentioned by chance in early investigations of marine habitat microbial communities. Such pioneering surveys did not use selective isolation processes or reliable diagnostic tests. Actinomycetes are thought to make up a minor percentage of the bacterial flora in marine ecosystems, with low numbers compared to those found in terrestrial and freshwater environments. Some researchers recognised actinomycetes as part of an indigenous marine microflora, whereas others saw them as wash-in components that only persisted as spores in marine and littoral sediments. The fact that the quantity of actinomycetes in maritime ecosystems decreases as the distance from land increases supports the latter viewpoint. The isolation of organisms from maritime areas far removed from the possibility of terrestrial contamination has been offered as evidence of a marine origin, although it is now clear that Thermoactinomycetes endospores may be transported extremely great distances by ocean currents. Small numbers of thermal actinomycetes were discovered in sediment collected from a depth of 4920 m in the Atlantic Ocean, 500 miles from shore. Actinomycetes were found to be abundantly distributed in the marine environment[8]. It has been reported that Streptomyces, Nocardia, and Micromonospora have grown on dead marine algae and contact slides suspended in the sea. The depth from which samples are taken affects the study of actinomycete dispersion in sediments. Silt is the most common marine source of actinomycetes, although they have also been found in water, sand, rocks, seafood, marine plants, mangrove sediment, and deep sediment. Agar, alginates, cellulose, chitin, oil, and other hydrocarbons have all been documented to be decomposed by actinomycetes from marine sources. They have also been linked to the degradation of wood submerged in water. Arthrobacter, Brevibacterium, Corynebacterium, and Nocardia are among the microorganisms crucial in the breakdown of petroleum hydrocarbons in aquatic settings, according to Atlas (1981). Under laboratory conditions, actinomycetes have been found to breakdown cellulose, starch, and lignin in seawater[9].

CHARACTERISTICS OF ACTINOMYCETES

Heterotropic is a characteristic of actinomycetes. While some are parasitic or mutualistic with other plants and animals the majority are strict saprophytes, others are not. Actinomycetes are typically assumed to contribute to the recycling of nutrients.

Actinomyces is an anaerobic organism, while some others are aerobic. Frankia species require very specific incubation and growing conditions[6]. While actinomycetes are known to grow in commonly used laboratory bacteriological media such as nutrition agar, trypticase agar, blood agar, brain heart infusion agar, and starch casein agar, specific media are required to enable the differentiation and development of distinct spores and colours in sporoactinomycetes.

Some of these media must be created in the lab using colloidal chitin, soil extracts, and plant decoctions because they are not commercially accessible. *Streptomyces* species can transform from pale, glossy, hard colonies on nutrition agar into bright yellow colonies with powdery white aerial mycelium and spirals of arthrospores when they are subculture on a more suited growth medium, like oatmeal or inorganic salts starch agar. As spores or mycelium fragments proliferate, they develop into hyphae that penetrate the agar (substrate mycelium) and hyphae that branch and adhere to one another on the agar's surface to form a thick, leathery colony. The density and uniformity of the colony depend on the composition of the medium. *Nocardioform actinomycetes* produce soft or friable colonies as a result of the hyphae disintegrating into rods and cocci[7].

In some strains of Streptomyces, a hydrophobic sheath that extends upward away from the colony covers the colony in loose, upright hyphae (aerial mycelium). These hyphae change from white to a variety of colours when spore development starts. Colonies can be distinguished from more typical bacterial colonies by their powdery or velvety appearance. The aerial mycelium of *Streptomyces* species has spore chains that are absent from the substrate mycelium. These spores are called arthrospores, which are just segments of regular hyphae with a thicker spore wall and a hydrophobic sheath that may or may not have spin or hair[10].

NOVEL TECHNIQUES FOR THE ISOLATION OF ACTINOMYCETES

New screening systems have been found to lead to the discovery of previously undiscovered and valuable natural compounds. The isolation of actinomycetes from natural mixed microflora is difficult due to their slow development compared to other soil bacteria.

There are five main processes for the isolation of industrially significant actinomycetes:

1. *Substrate selection*. It has proven possible to isolate actinomycetes from both freshwater and marine habitats. There must be some differences between species living in terrestrial and aquatic environments. During the screening process, several antagonistic actinomycetes, such as those that produce xanthomycin, were isolated from shallow sea actinomycetes more frequently than from terrestrial soil. Few of these actinomycetes were found to be novel, producing fresh antibiotics or chemical compounds with physiological activity under carefully monitored conditions. Because

of this, isolating actinomycetes from marine settings offers a different way to find new actinomycetes and antibiotics.

2. *Re-heat treatment*. A pretreatment that makes it possible to isolate an actinomycete component that is often seldom or absent from soils. One instance is the rehydration of freshwater leaf litter, which has resulted in the production of many actinoplanetes, including the newly discovered Cupolomyces genus. The airborne spores of the majority of actinomycetes can endure both wet and dry heat and are resistant to desiccation. Such modest temperature treatments significantly eliminate Gram-negative bacteria. With the aid of a selective medium, a combination of drying and mild heat treatments can be used to separate bioactive actinomycetes from marine sediments. By implementing pre-treatment techniques such as dry heating, specialised growth media, and extended incubation periods, a range of novel species belonging to Actinomadura, Microbispora, Microtetraspora, Streptosporangium, Thermomonospora, and Thermoactinomyces were successfully isolated[11]. Various actinomycetes, bacteria, and fungi that are prevalent in soil are used to test a wide range of antibiotics. Nystatin (50 g/ml), acetidione (50 g/ml), polymyxin B sulphate (5 g/ml), and sodium penicillin (1 g/ml) are frequently used to produce selective growth of actinomycetes on soil dilution plates. Recently, differences in antibiotic sensitivity have been used to increase the media's selectivity for particular actinomycetes. Rubomycin, bruneomycin, streptomycin, kanamycin, and rifampicin for *Actinomadura* spp. Novobiocin for *Thermoactinomycetes* spp.

3. *Selective media*. Actinomycetes have benefited by the addition of bacteriostatic and fungistatic agents to isolation media, such as phenol and sodium propionate, which suppress the growth of bacteria and moulds. However, such alterations frequently permit the proliferation of contaminants at permissible levels, and they may also inhibit actinomycetes at higher levels. Chitin agar with mineral salts is more effective than chitin agar without mineral salts for isolating actinomycetes from water. Chitin agar outperformed other mediums in terms of selectivity for isolating actinomycetes from water and soil[12].

4. *Incubation*. Actinomycetes that produce antibiotics often prefer temperatures between 25 and 30°C. Thermopiles are maintained at 40–45°C, while psychrophiles are maintained at 4–10°C. Usually, isolation plates need to be incubated for 7 to 14 days. In the past, longer incubation periods have gone unnoticed because slow-growing actinomycetes are not suitable candidates for industrial fermentation. On the other hand, early growth of some bacterial species can modify the nutritional environment of the isolation plate by supplying growth factors. To isolate new actinomycetes, the incubation time may be increased by one month.

5. *Colony selection*. Colony selection is the procedure that takes the longest. Depending on the goals of the screening program. There may be significant colony duplication. There is a need for more rational techniques for isolating microbes. Most scientists

now choose potential colonies with a stereomicroscope and transfer growth with a sharp cocktail stick made of wood. The rough wooden points hold enough spores or hyphal fragments to guarantee a successful transfer, and little colonies can be found and chosen. The location of the sample collection, knowledge of the secondary metabolite of an isolate, objective enrichment techniques, and objective culture media compositions would all contribute to the isolation of novel and promising isolates[13].

IMPORTANCE OF ACTINOMYCETES IN BIOTECHNOLOGY

Actinomycetes' significance in biotechnological applications is a natural outcome of their extensive metabolic diversity and extensive environmental interaction. Prokaryotic species classified as "actinomycetes" have unique morphological, cultural, biochemical, and physiological traits. This company has the ability to produce antimicrobials, enzyme inhibitors, immunomodifiers, enzymes, and growth promoters for both plants and animals[13].

1. *Antibiotics*. The most abundant manufacturers of antibiotics are actinomycetes. Actinomycetes are responsible for two-thirds of today's antibiotics. Anthracyclines, aminoglycosides, -lactams, chloramphenicol, macrolides, tetracyclines, nucleosides, peptides, and polyethers are all essential antibiotics produced by actinomycetes. Actinomycete antibiotics were virtually entirely restricted to Streptomyces until 1974. Rare actinomycetes such as Actinomadura, Actinoplanes, Ampullariella, Actinosynnema, and Dactylosporangium have recently been investigated in the search for novel antibiotics. Antibiotic generating actinomycetes are screened via target focused screening. Molecular biological approaches have aided in the discovery of novel antibiotics from actinomycetes on a broad scale. Many elements of basic study on actinomycetes have been pushed by their usefulness in industrial biosynthesis[9].

2. *Xenobiotic transformation*. Xenobiotic transformation is defined as the structural modification of non-metabolic components that occur in an organism's chemical environment. Oxidative, reductive, hydrolytic, dehydration, and condensation are the most common xenobiotic transformation processes. Actinomycetes' ability to undertake a variety of microbiological conversions of organic molecules is a key factor in the sophisticated biodegradation of contaminants in soil and water. Members of the genera Nocardia and Streptomyces are capable of performing extremely selective chemical changes on complex natural and manmade substances. Aromatic hydrocarbons are degraded by hydroxylation in Nocardia strains. Actinomycetes have the ability to hydroxylate aliphatic hydrocarbon chains in the terminal and subterminal positions, which is followed by the transformation of the chains. Certain insecticides can be degraded by actinomycetes. Nocardia strains identified from soil decomposed the herbicide dalapon, 2,2-dichloropropionic acid[14].

3. *Immunomodifiers*. Filtrates from Actinomycetes culture have produced low molecular weight compounds that enhance immune responses. Immunomodifiers are

the term for such substances. Inhibitors of enzymes found on the surface of immune cells may attach to these cells and enhance immunological responses. Streptomyces olivoreticuli bestatin, Streptomyces olivoreticuli amastatin Phenicine from *Streptomyces lavendulae*, species ME 98-M-3, improved immunological responses in mice. Interleukin-2 synthesis, mixed lymphocyte reaction, interferon, cytotoxic-T cells, and platelet activating factor-C induction are all inhibited more effectively by immunosuppressive drugs like FR-900506, which is generated by *Streptomyces tsukubensis* sp.[15]

4. *Biosurfactant*. A biosurfactant is a surface-active chemical produced mostly by microorganisms by living cells. The term "biosurfactant" has been applied to any substance produced by microorganisms that has some effect on surfaces. Surface tension measurements are used in the evaluation of biosurfactants. Surfactant and emulsifier are frequently used interchangeably in the literature. Biosurfactants have a number of advantages over chemically manufactured surfactants. They are very selective, biodegradable, and less harmful. They work in a wide range of temperature, pH, and salinity conditions. They are simple to make using low-cost, renewable feedstocks. Bioemulsifier production is dominated by actinomycetes. Wagner and his colleagues have examined trehalose dimycolates generated by *Rhodococcus erythropolis* thoroughly[16,17].

5. *Enzyme inhibitors*. Low-molecular-weight enzyme inhibitors are made by actinomycetes. Typically, it is believed that microorganisms produce low-molecular-weight chemical molecules called antibiotics[18]. Since then, more than 60 inhibitors have been found, including leupreptins, which obstruct papain, plasmin, and trypsin. Antipain inhibits the digestive enzymes papain, chymotrypsin, trypsin, and cathepsin B. Researchers are looking into enzyme inhibitors as a potential cancer treatment. Reverse transcriptase is inhibited by revistin, an enzyme inhibitor found in *Streptomyces* species. Streptomyces produces streptonigrin and retrostatin, which inhibit reverse transcriptase. Alistragin, discovered in *Streptomyces roseoviridis* culture filtrates, inhibits carboxypeptidase B. S. tanashiensi produces phosphoamiden, which inhibits metallo-proteases[19].

ENZYMES

1. *Amylase enzyme*. The hydrolysis of internal a-1, 4-O-glycosidic links in polysaccharides is catalysed by amylases, starch-degrading enzymes that preserve an anomeric structure in the finished products. For activity, structural stability, and stability, calcium ions (Ca^{2+}) are necessary for the majority of metalloenzymes, including α-amylases. They belong to the family of glycoside hydrolases (GH-13). One of the most important industrial enzymes is amylase, with applications ranging from the manufacture of cyclodextrins for the pharmaceutical sector to the conversion of starch to sugar syrup. These enzymes generate about 30% of the total amount of enzymes produced worldwide. Enzymes that hydrolyse starch and enzymes that alter or transglycosylate

starch are two subgroups of the α-amylase family. In the starch processing industry, enzyme hydrolysis is preferred to acid hydrolysis for a number of reasons, including reaction selectivity, product stability, reduced energy requirements, and the absence of neutralisation steps. There is a lot of interest in creating enzymes with higher qualities due to the expanding need for these enzymes in numerous industries, such as raw starch degrading amylases for industrial applications and inexpensive production processes[4].

2. *Lipase enzyme*. Lipases are hydrolases of carboxylic ester bonds. Triglycerides are broken down by lipases into glycerol, monoglycerides, fatty acids, and diglycerides. Plants, animals, moulds, and microorganisms all have lipases. Along with lipases, esterases can hydrolyse carboxylic esters bonds[20].

3. *Thermostable/alkalophilic enzymes*. The number of uses for thermostable lipases is rapidly expanding. Most of the research to far has focused on mesophilic producers. Numerous mesophile lipases are stable at higher temperatures. Proteins from thermophilic organisms have shown to be more stable than comparable proteins from thermophiles, making them more useful for biotechnological applications. The advantages of biocatalyst thermostability include increased reactivity (higher reaction rate, fewer diffusional restrictions), increased stability, higher process yield (increased solubility of substrates and products and favourable equilibrium displacement in endothermic reactions), lower viscosity, and fewer contamination issues. These advantages balance the drawbacks of stricter material specifications, more challenging post-reaction inactivation, and restrictions in the case of substrates or products that are labile. Thus, thermostable biocatalysts are highly desirable. Even psychrophiles have certain thermostable enzymes, which are seen in mesophilic and thermophilic species. Since thermophiles are a natural source of thermostable enzymes, it makes sense to assume that, by virtue of their biology, their proteins will be highly thermally stable. Numerous biotechnologically significant enzymes from the hyperthermophilic archaebacteria Pyrococcus furiosus and Thermotoga provide indisputable evidence for this[21–23].

4. *Gelatinase enzyme*. Gelatinase is a proteolytic enzyme found in biology and chemistry that degrades gelatin into smaller molecules (amino acids, polypeptides, and peptides) that may pass through cell membranes and be utilised by the organism. The culprit is pepsin. Several bacteria, including Serratia marcescens and Pseudomonas aeruginosa, express gelatinases in various ways. The human gelatinase genes are MMP2 and MMP9 (Ref. 24).

5. *Chitinase enzyme*. Chitinases are quite promising. The extraction of yeast and fungal protoplasts, the management of pathogenic fungi, the treatment of chitinous waste, the prevention of malaria transmission, and the production of pharmaceutically important chitooligosaccharides and N-acetyl d-glucosamine are all processes that use chitinolytic enzymes. The presence and structure of chitin, the types and sources of chitinases, their mechanisms of action, chitinase[25]. The most important commercial

source of chitin and chitosan is shrimp trash. Chitin is found in the exoskeleton of crustaceans, insects, and fungus; it is the second most common natural polysaccharide after cellulose. Biomaterials like chitin and chitosan are exceedingly adaptable and promising. Unacetylated chitin, the more useful and exciting biologically efficient monomer, is the source of chitosan, a bioactive polymer[26]. It is possible that despite being biodegradable, this has undergone chemical modification to yield derivative compounds that have a range of biological and biotechnological uses. Although chitin and chitosan both possess antiplatelet and antiglycation characteristics, chitosan (a chitin derivative) was more successful in biological applications than chitin[27].

APPLICATIONS OF ENZYMES

Use of α-amylase enzyme. The first enzymes to be synthesised and commercialised commercially were amylases. The first industrial synthesis of α-amylase from *A. oryzae* was created by Dr. J. Takamine and was known as "Taka diastase" because it was used as a digestive aid. The enzyme market was worth $2 billion worldwide in 2004. It is anticipated that annual growth will average 3.3%. About 40% of all enzymes are carbohydrases, which include amylases, isomerases, pectinases, and cellulases. The food and beverage industries consume 90% of the generated carbohydrases. Approximately $11 million is predicted to be spent annually on the global market for – amylases. Every year, amylases from B. licheniformis and Aspergillus sp. produced about 300 tons of pure enzyme protein[20–22].

Protease enzyme application. The most typical industrial uses for alkaline proteases are covered in the following section – food and feed industry. The food industry has long exploited microbial proteases for a number of uses. Alkaline proteases have been used to create protein hydrolysates with great nutritional value. In addition to being used in infant food formulations, some therapeutic dietary supplements, and the fortification of fruit juices and soft drinks, protein hydrolysates also play a vital role in controlling blood pressure. The capacity of proteases to hydrolyse proteins, which is their main function, has been used to produce protein hydrolysates rich in nutrients. To create hydrolysates from a range of natural protein substrates, alkaline proteases are used.

Leather industry: Traditional leather production processes use hydrogen sulphide and other chemicals, which pollute the environment and pose safety risks. For environmental reasons, an enzymatic approach to leather biotreatment is preferred since it offers various advantages, such as easy control, speed, and waste reduction, making it eco-friendly. In the leather industry, alkaline proteases having elastolytic and keratinolytic activity can be utilised. Proteases are used in the soaking, dehairing, and bating stages of skin and hide preparation. The enzymatic treatment removes unwanted colours, expands the skin area, and produces a clean hide. Batting has long been thought to be an enzymatic process involving pancreatic proteases. However, microbial alkaline proteases have recently gained popularity. Alkaline proteases speed

up the dehairing process because alkaline circumstances induce the hair follicle protein to expand, making it easier to remove the hair. Unhairing sheepskin using *B. subtilis* IIQDB32 alkaline protease was reported.

Photographic industry: Alkaline proteases are important in the bioprocessing of discarded X-ray or photographic films in order to recover silver. The gelatin layer of these waste films contains 1.5–2.0% silver by weight, making them a valuable source of silver for a variety of applications. Traditionally, this silver has been collected by burning the films, which pollutes the environment. Furthermore, polyester base film cannot be recovered using this procedure. Because the silver is attached to gelatin, proteolytic procedures can remove the silver from the protein layer. Enzymatic gelatin hydrolysis not only aids in the extraction of silver, but also allows the polyester film basis to be recycled. The alkaline protease from Bacillus sp. B21-2 was used to enzymatically hydrolyse the gelatin layer of X-ray films to release silver particles in 30 min at 50–60°C. Bacillus sp. B18 and Bacillus coagulans PB-77 alkaline proteases were also effective in disintegrating the gelatinous layer on discarded X-ray films, allowing the silver to be retrieved.

Medical applications: Alkaline proteases have also been employed to generate medical goods. For example, the elastolytic activity of *B. subtilis* 316M was used to make elastoterase, which was used to treat burns, purulent wounds, carbuncles, furuncles, and deep abscesses. Moreover, the genus Bacillus has produced a number of direct-acting fibrinolytic enzymes, such as subtilisin DJ-4 (*Bacillus amyloliquefacien*), subtilisin DFE (*B. amyloliquefacines*), and subtilisin CK (*Bacillus* sp. strain CK-11-4)[28].

Silk degumming: The silk industry is one of the least researched sectors for the use of proteases, with only a few patents documenting the use of proteases for silk degumming. Sericin, which accounts for around 25% of the total weight of raw silk, coats the raw silk threads' perimeter, giving the silk fibres their rough texture. The sericin is traditionally removed from the inner core of fibroin by shrink-proofing and twist-setting the silk strands with starch.

Enzyme preparations: Because the technique is often costly, an alternative option recommended is degumming the silk prior to dyeing with enzyme preparations such as protease. The silk-degumming efficacy of an alkaline protease from Bacillus sp. RGR-14 was investigated in our lab recently, and the results were examined gravimetrically (fibre weight decrease) and by scanning electron microscopy.

Detergent industry: Enzymes have long piqued the interest of the detergent industry due to their capacity to aid in the removal of proteinaceous stains and to provide unique benefits not available from traditional detergent technologies[19,29].

Pharmaceutical business: Biocatalysts have significant advantages over chemical synthesis in the pharmaceutical industry, justifying the increased need for enzymes. Enantio- and regioselectivity; moderate conditions that minimise isomerisation, racemisation, epimerisation, and rearrangement processes; overexpression of enzymes; reuse of immobilised biocatalysts; process economy; and mutagenesis of enzymes

for specific roles are only a few of the benefits. The pharmaceutical industry currently uses lipases' ability to resolve racemic mixtures via creation of a single enantiomer for medication manufacturing. In fact, only one enantiomer of a medicine is responsible for the desired therapeutic action, and optically pure pharmacological products have milder or fewer adverse effects than racemic mixes. Some of the lipases can be employed to make enantiopure molecules such alcohols, amides, carboxylic acids, and esters. Anti-inflammatory medications (ibuprofen, naproxen), anticancer treatments (lobucavir), hypertension drugs (captopril), anti-cholesterol therapies (squalene synthase inhibitor), anti-Alzheimer disease drugs ([S]-2-pentanol), and vitamin A all contain these compounds.

Racemic chemical resolution: The anticancer medicine Taxol® (paclitaxel) is an antimitotic agent that prevents microtubulin depolymerisation during mitosis. This medication is used to treat ovarian cancer as well as metastatic breast cancer. Taxol® sells for around $1 billion each year in the United States. Paclitaxel was first isolated and refined in a relatively low yield from the bark of the yew Taxus brevifolia. Paclitaxel can also be made by connecting baccatin III (paclitaxel without the C-13 side chain) or 10-deacetylbaccatin II ([10-DAB], paclitaxel without the C-13 side chain and the C-10 acetate) to the C-13 paclitaxel side chains in a semisynthetic method.

Baccatin III and 10-DAB can be produced from renewable sources such needle extract, shoot extract, and young Taxus cultivars, avoiding the need to cut down yew trees. Enantioselective hydrolysis of racemic acetate-cis-3-(acetoxy)-4-phenyl-2-azetidione to the matching (3S)-alcohol and the intact required (3R)-acetate yielded the C-13 paclitaxel side chain. The lipase PS-30 from *Pseudomonas cepacia* or the Bristol-Myers Squibb (BMS) lipase from *Pseudomonas* sp. SC 13856 catalysed the hydrolysis. Before use, both lipases were immobilised on Accurel MP1000 (a porous polypropylene powder). For (3R)-acetate, an enantiomeric excess of > 99.5% was produced, as well as reaction yields of > 48% (highest theoretical yield: 50%). Using immobilised BMS lipase and Lipase PS30, the process was scaled up to 75 and 150 l, respectively.

Resolution of racemic ester: Another efficient and significant industrial lipase application is diltiazem, a calcium channel blocker with an annual market of around US $85 million in 1996 and 22.5 billion Yen in 2002. Each year, Tanabe produces 50 t of diltiazem. An important step in the production of an intermediate needed for the synthesis of diltiazem is the resolution of racemic epoxy esters. This enantiospecific hydrolysis is carried out by a lipase from *Serratia marcescens*.

CONCLUSIONS

Marine actinomycetes and their biotechnological applications have been previously documented, these microorganisms have not received significant attention in research, and their role in the microbial community of marine habitats has only been briefly explored. Additionally, there is a dearth of knowledge regarding the prevalence and

distribution of actinomycetes in the mangrove environment (which is one of the most prolific coastal ecosystems). According to recent investigations using culture-dependent and culture-independent techniques, indigenous marine actinomycetes are widespread in many marine settings and can be discovered in the oceans. Incredibly diverse and uncommon marine actinomycetes are found in watery habitats. Materials gathered from various maritime environments and situations, where various kinds of secondary metabolites are produced, led to the isolation of novel actinomycetes, emphasising the potential for these microorganisms to provide a wealth of valuable bioactive compounds. Further research into marine actinomycetes and their biotechnological potential could yield significant benefits in a variety of fields, including medicine, agriculture, and biotechnology.

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