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Hypersaline Actinomycetes and Their Biological Applications

Mariadhas Valan Arasu, Galal Ali Esmail and Naif Abdullah Al-Dhabi

Abstract

Actinomycetes are the potential sources of novel metabolites, therapeutic compounds, enzymes, and other chemicals. Among them, the applications of halophilic actinomycetes toward the medically and industrially important metabolites and enzymes are gaining increasing attention by the scientific community. A large number of novel compounds and enzymes from halophilic actinomycetes have been isolated and characterized from various geographic regions around the world. In this chapter, occurrence, characterization, halotolerant mechanisms, medical importance, metabolites, enzymes, and industrial applications of halophilic actinomycetes are discussed. Halophilic actinomycetes may also serve as good models for the production of important metabolites and enzymes with respect to stress response.

Keywords: Halophilic actinomycetes, occurrence, metabolites, enzymes, applications

1. Introduction

Actinomycetes are the most valuable microorganisms for the production and synthesis of economically important therapeutic compounds and antibiotics. They are the source for the production of about more than 50% of discovered bioactive compounds, including antitumor agents, antibiotics, enzymes, and immunosuppressive agents [1, 2]. Most of these bioactive secondary metabolites were isolated from terrestrial actinomycetes; however, in recent
years, the rate of discovery of new bioactive compounds has decreased. Hence, it is crucial
that new groups of actinomycetes from unexplored or underexploited environments should
be exploited optimally to obtain novel bioactive secondary metabolites [3]. Unexplored re-
gions of high salt deposited areas, conditions closely related to the marine water regions, re-
gions with increased levels of pH, and low oxygen were the suitable places to isolate
halophilic actinomycetes. Actinomycetes recovered from halophilic and unexplored regions
attracted the interest of researchers because of their applications in the medical and biotech-
nological fields [4]. This chapter deals with the characterization methods for the identifica-
tion of halophilic actinomycetes and their applications

2. Occurrence of halophilic actinomycetes

Marine sediment, soil, water, contaminated regions on the seashore, salt lakes, saline soils,
alkaline–saline habitats, brines, and other regions are good sources for the selection of novel
halophilic actinomycetes. Recent literature claimed that the saline regions contain many sig-
nificant uncultured actinomycetes [5]. In particular, actinomycetes such as *Streptomyces phar-
namarensis*, *Prauserella halophila*, *Pseudonocardia* sp., *Micromonospora* sp., *Marinactinospora thermotolerans*, *Microbacterium* sp., *Nocardiopsis xinjiang-
gensis*, *Salinibacterium* sp., *Salinactinospora qingdaonensis*, *Aeromicrobium* sp.,
*Marinactinospora arenicola*, *Actinomadura* sp., *Saccharopolyspora* sp., *Streptosporangium* sp., *Actinopolyspora algeriensis*, *Marinophilus* sp., *Streptomonospora halophila*, *Verrucosispora* sp., *Gordonia* sp., and *Nonomuraea* species were re-
covered and characterized from hypersaline regions [6–10]. Also, it was quoted that the
characterization of novel halotolerant actinomycetes was specific with respect to their mor-
phological, biochemical, physiological, and molecular level identification.

3. Identification and characterization of halophilic actinomycetes

In general, different pretreatment methods such as incorporation of specific antibiotics were
used to prevent the growth of bacteria and fungi, and alteration in the composition of the
cultivation medium was specific for the isolation of rare actinomycetes. However, humic
acid and different concentrations of vitamin agar medium were the best base for the selec-
tion of rare halophilic actinomycetes. Humic acid strengthens the morphological identifica-
tion and characterizes the spore chain of the actinomycetes by preventing the production of
diffusible pigments in the cultivation medium. A substantial part of the microbes in envi-
ronmental samples are not cultivable and therefore cannot be identified by methods based
on culturing of the strains. However, protocols based on molecular techniques are not de-
pendent on the viability of the microbes, and molecular methods for the amplification of
specific genes such as 16S rRNA and *rec A* can be designed to work at different levels of spe-
cificity for the detection of whole groups of actinomycetes. Polymerase chain reaction
(PCR)-based methods for the detection and identification of microbes are widely used in the
cultivation of industrially important microbes [11]. Applications in environmental microbi-
ology, especially in the soil environment, are also increasing [12]. With currently used cultivation methods, only a small part of the halophilic actinomycetes diversity is detected. The cultivability values ranged from 0.001% to 15%, depending on the cultivation area and the method of cultivation medium [13]. On the other hand, PCR amplification of 16S rRNA genes from environmental samples has revealed that 7–64% of the amplified sequences originated from uncultured actinomycetes [14]. Comparisons of the amounts of total and cultivable microbes in the indoor environment have shown that the cultivable part of the microbial community ranges from 1% to 10% [15]. Therefore, careful attention must be paid in the identification of the rare halophilic actinomycetes.

4. Halotolerance mechanism

Halophilic actinomycetes require higher concentrations of salt for their growth and are classified into moderate (15% NaCl) and extreme (30% NaCl) halophiles. They survive through two mechanisms – “high-salt-in” and “low-salt, organic-solutes-in” – for the protection of intercellular proteins in the presence of salts similar to potassium chloride and production of organic acids, which will directly alter the intracellular enzyme levels. To adjust to the hyper-salt conditions, halophilic cell membranes are composed of main adaptive systems that block NaCl from penetrating into the cells, such as the accumulation of inorganic salts and the water-soluble low-molecular-weight organic compounds. Inorganic salts in the form of (Na⁺, K⁺, Cl⁻) are mainly involved in adjusting the osmotic potentials, and the organic salts in the form of small solutes or electrolytes are concerned with the balance of the intracellular salt levels [16]. These two mechanisms are mainly involved in the maintenance of the cell structure; therefore, the cells thrive in stress conditions such as high salt [17].

5. Importance of halophilic actinomycetes

Actinomycetes identified in the halophilic regions such as salt lakes, salterns, solar salts, and subsurface salt formation have to cope up with the osmotic stress were an infinite pool of novel chemical molecules with increasing importance for several biotechnological applications in different fields. Traditionally, halophiles have been used in the food and nutraceutical industries for the fermentation of soy and fish sauces and β-carotene production; also they have been recently used in many novel and unique molecules such as compatible solutes, biopolymers or carotenoids, enzymes, biodegradable plastics, biosurfactants, bioemulsifiers, and bacteriorhodopsins for molecular biotechnology applications [18, 19]. Among the valuable products, enzymes obtained from these organisms, mainly involved in the processing of food, act as a catalyst in the bioremediation process and other biosynthetic processes. In addition to the enzymes, highly sensitive chromoproteins derived from halophiles act as a biocatalyst and light-sensitive neurological probe for the treatment of blindness [20]. Ectoine and hydroxyectoine obtained from the halophilic actinomycetes are commercially used as protective and stabilizing agents for mammalian cells [21]. Ectoine derived from
Halomonas boliviensis fermentation reduces the DNA lesions induced by visible and UVA-visible lights. Besides, halophiles attracted many researchers for the development of sustainable energy production to minimize the effects of global warming. Because halophiles have superior qualities such as high tolerance levels of the enzymes toward salts and temperatures, stability in the presence of organic solvents, secretion of therapeutic compounds, and antimicrobial properties, in many applications, studies of halophilic bacteria have developed rapidly and significant advances have been made recently [22].

6. Metabolites produced by halophilic actinomycetes

In the last two decades, halophilic actinomycetes have gained significant importance as a new, promising source for novel bioactive compounds that can be used for drug development. Halophilic actinomycetes may produce a variety of bioactive compounds, which have a wide range of biological activities, including antibacterial, antifungal, antiviral, and other bioactive compounds [23]. The vast majority of bioactive compounds that isolated from halophilic actinomycetes are derived from the genus Streptomyces, whose species are very widely distributed in nature and cover around 80% of the total produced antibiotics [24].

7. Antibacterial

An antibacterial is an agent that may either inhibit the bacterial growth or kill the bacteria. The urgent need to find novel antibacterial agents has increased during the last decade owing to the increase of emerging multidrug-resistant bacteria. The discovery of novel antibiotics that have a potent effect against resistant pathogenic bacteria is an important aspect of antibiotics research today. The diversity of natural products makes it one of the most important sources for novel structures, which have been noticed to possess useful biological activities. Several studies are oriented toward the isolation of new actinomycetes from different habitats in the context of the search for novel antibiotics from new sources representing one of these habitats [25].

Generally, the antibacterial activity of halophilic actinomycetes from marine environments is extensively studied. Nevertheless, the exploitation of halophilic actinomycetes as a source for the discovery of novel antibiotics is still at an early stage, despite that numerous novel antibacterial compounds were isolated during the last few years (Table 1). Arenimycin (Fig. 1a) is a novel antibacterial compound isolated from the extreme halophilic actinomycete Salinispora arenicola. This compound was classified as a new antibiotic based on the novel structure, which belongs to the benzo [α] naphthacene quinone class of antibiotics. Arenimycin has proven to show a potent antibacterial activity against a panel of drug-resistant human pathogens such as rifampin- and methicillin-resistant Staphylococcus aureus. Arenimycin is a representative of the first report of this class of antibiotics from marine actinomycetes [26]. Abyssomicin C (Fig. 1b) is another example for novel antibacterial compounds extracted...
from the halophilic actinomycete *Verrucosispora* sp. [27]. Abyssomicin C was derived with other two novel structures that belong to the polycyclic polyketides abyssomicin B and abyssomicin D. Among these three compounds, abyssomicin C strongly exhibits antibacterial activity against Gram-positive bacteria, including multiresistant clinical isolates of *Staphylococcus aureus*. The mode of action of this new antibiotic is based on the inhibition of para-aminobenzoic acid biosynthesis resulting in the inhibition of the folic acid biosynthesis pathway.

![Chemical structure of some novel antibacterial compounds produced by halophilic actinomycetes](image)

**Figure 1.** Chemical structure of some novel antibacterial compounds produced by halophilic actinomycetes [26, 27].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-Dihydroxy-2-(3-hydroxybutyl)-9,10-anthraquinone 9,10-anthrac</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>1-Hydroxy-1-norresistomycin</td>
<td><em>Streptomyces chinaensis</em></td>
</tr>
<tr>
<td>Abyssomicins</td>
<td><em>Verrucosispora</em> sp.</td>
</tr>
<tr>
<td>Arenimycin</td>
<td><em>Salinispora arenicola</em></td>
</tr>
<tr>
<td>Bisanthraquinone</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>Bonactin</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>Caboxamycin</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>Chloro-dihydroquinones</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>Diazepinomincin</td>
<td><em>Micromonospora</em> sp.</td>
</tr>
<tr>
<td>Essramycin</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>Frigocyclinone</td>
<td><em>Streptomyces griseus</em></td>
</tr>
<tr>
<td>Glaciapyroroles</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>Gutingimycin</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
</tbody>
</table>
Compound Source
Helquinoline Janibacter limosus
Himalomycins Streptomyces sp.
Lajollamycin Streptomyces nodosus
Lincomycin Streptomyces lincolnensis
Lynamicins Marinispora sp.
Marinomycins Marinispora sp.
Marinopyrroles Streptomyces sp.
Proximicins Verrucomicrobiaceae sp.
Resistoflavin methyl ether) Streptomyces sp.
Tirandamycins Streptomyces sp.
TP-1161 Nocardiopsis sp.

The listed compounds and the strains were obtained from the report of Subramani and Aalbersberg, (2012) [36].

Table 1. Novel antibacterial compounds produced by halophilic actinomycetes

8. Antifungal

The number of antifungal agents available for controlling fungal infections is still limited in comparison to antibacterial agents, and their use is still risky owing to toxicity and side effects [28]. Therefore, studies are still being conducted to isolate and identify novel antifungals that are potentially effective against pathogenic fungi [23]. Halophilic actinomycetes from marine environments are useful biological tools for the discovery of novel antifungal substances against fungi. Few studies reported the isolation of antifungal agents from halophilic actinomycetes. *Streptomyces* is the main source for antifungal agents (Table 2) which have been isolated from marine habitats.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azalomycin F4a 2-ethylpentyl ester</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>Bonactin</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>Chandrananimycin</td>
<td><em>Actinomadura</em> sp.</td>
</tr>
<tr>
<td>Daryamides</td>
<td><em>Streptomyces</em> sp.</td>
</tr>
<tr>
<td>N-(2-hydroxyphenyl)-2-phenazinamine (NHP)</td>
<td><em>Nocardia dassonvillei</em></td>
</tr>
</tbody>
</table>

The listed compounds and the strains were obtained from the report of Subramani and Aalbersberg, (2012) [36].

Table 2. Novel antifungal agents produced by halophilic actinomycetes
Chandrananimycin A (Fig. 2a) is a novel antifungal substance produced by marine Actinomadura sp. strain M048. Chandrananimycin A exhibited strong, effective antifungal activity against *Mucor miehei*. It exhibits other biological activities as an antibacterial, anticancer, and antialgal agent against the microalgae [29]. N-(2-hydroxyphenyl)-2-phenazinamine (NHP) (Fig. 2b) is a new antibiotic produced by halophilic actinomycetes isolated from the sediment sample *Nocardia dassonvillei* collected from the Arctic Ocean [30]. The new antibiotics possess potent antifungal activity against *Candida albicans*, with an MIC of 64 µg/ml.

![Figure 2. Chemical structure of novel antifungal isolated from halophilic actinomycetes [30].](image)

### 9. Antiviral

Natural products represent the main source for discovering new/novel chemical structures that are used for the treatment of infections. The vast majority of secondary metabolites produced by microbes that have been developed for controlling microbial infections are directed against bacterial and fungal infections but not against viral infections. To date, antiviral agents have been isolated from natural products that are limited, and the studies in this field are few [31]. The antiviral agents available commercially in pharmacological markets are over 40 compounds, including those being tested as promising antiviral agents or alternative antiviral medicines [32]. Potential substances that possess antiviral activity have been isolated from halophilic microorganisms from marine environments. EPS-1 and EPS-2 are antiviral agents produced by *Bacillus licheniformis* and *Geobacillus thermodenitrificans*, respectively, targeting the replication of herpes simplex virus type 2 (HSV-2) [33]. Cyanovirin-N was obtained from the halophilic cyanobacteria *Nostoc ellipsosporum*. Cyanovirin-N has antiviral activity against HIV-1 and HIV-2 by targeting the replication [34]. However, halophilic actinomycetes produce several bioactive compounds, the compounds derived from halophilic actinomycetes as antiviral agents are limited. Antimycin A is an antiviral substance produced by *Streptomyces kaviengensis* isolated from marine sediments collected from the coast of New Ireland, Papua New Guinea. Antimycin A has potent antiviral activity against western equine encephalitis virus and a wide range of RNA viruses in cultured cells, includ-
ing members of the Togaviridae, Flaviviridae, Bunyaviridae, Picornaviridae, and Paramyxoviridae families. Benzastatin C (Fig. 3) is a 3-chloro-tetrahydroquinolone alkaloid extracted from the halophilic actinomycete *Streptomyces nitrosporeus* [35]. This compound exhibited antiviral activity against herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and vesicular stomatitis virus (VSV), respectively, in a dose-dependent manner with EC50 values of 1.92, 0.53, and 1.99 µg/mL.

![Chemical structure of benzastatin C produced by *Streptomyces nitrosporeus* [35].](image)

10. Therapeutic compounds

Diseases constitute a significant threat in the life of humans; over 30,000 diseases have been described clinically. Nevertheless, only less than one third of these can be treated [36]. There is an urgent need to obtain new therapeutic agents to cover this medical requirement. Natural products are able to fulfill medical needs through the discovery of novel therapeutic compounds [37]. In this regard, investigations on halophilic actinomycetes during recent years have led to the development of numerous isolated therapeutic substances (Table 3), including anticancer, antitumor, anti-inflammatory, antioxidant, and antimalarial substances [38]. Salinosporamide A (Fig. 4a) is a novel anticancer substance isolated from the marine actinomycete *Salinispora tropica*. Its chemical structure is bicyclic beta-lactone gamma-lactam. The mode of action of salinosporamide A is the inhibition of proteasome, which leads to induced apoptosis in multiple myeloma cells [39]. Nereus Pharmaceuticals, Inc., has developed salinosporamide A under the name NPI-0052 for the treatment of cancer in humans, which represents the first clinical anticancer agent candidate for the treatment of cancer produced by halophilic actinomycetes [36]. Lodopyridone (Fig. 4b) is an anticancer agent obtained from the marine actinomycete *Saccharomonospora* sp. [40]. Lodopyridone exhibited anticancer activity against the human colon adenocarcinoma cell line HCT-116 with an IC50 of 3.6 µM. Cyclomarin A is a novel anti-inflammatory agent isolated from halophilic *Streptomyces* sp. It is a cyclic heptapeptide and possesses potent anti-inflammatory activity in both
in vivo and in vitro assays. Trioxacarcin is a new antimalarial substance isolated from marine Streptomyces sp. [41]. Trioxacarcin has been tested for antimalarial activity. Results showed extremely high antiplasmodial activity against the pathogen of malaria in comparison to the artemisinin drug, the most effective drug against malaria. Actinosporins C and D are novel antioxidants produced by the sponge-associated actinomycete Actinokineospora sp. strain EG49 isolated from the marine sponge Spheciospongia vagabunda [42]. Actinosporins C and D showed (at 1.25 µM) a significant antioxidant and protective capacity from the genomic damage induced by hydrogen peroxide in the human promyelocytic (HL-60) cell line.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biological activity: anticancer</strong></td>
<td></td>
</tr>
<tr>
<td>1-Hydroxy-1-norresistomycin</td>
<td>Streptomyces chianaensis</td>
</tr>
<tr>
<td>3,6-Disubstituted indoles</td>
<td>Streptomyces sp.</td>
</tr>
<tr>
<td>Caprolactones</td>
<td>Streptomyces sp.</td>
</tr>
<tr>
<td>Chinikomycins</td>
<td>Streptomyces sp.</td>
</tr>
<tr>
<td>IB-00208</td>
<td>Actinomadura sp.</td>
</tr>
<tr>
<td>Lodopyridone</td>
<td>Saccharomonospora sp.</td>
</tr>
<tr>
<td>Marinomycins A-D</td>
<td>Marinispora</td>
</tr>
<tr>
<td>Mechercharmycins</td>
<td>Thermoaactinomycetes sp.</td>
</tr>
<tr>
<td>Salinosporamide A</td>
<td>Salinispora tropica</td>
</tr>
<tr>
<td>ZHD-0501</td>
<td>Actinomadura sp.</td>
</tr>
<tr>
<td><strong>Biological activity: antitumor</strong></td>
<td></td>
</tr>
<tr>
<td>1,8-Dihydroxy-2-ethyl-3-methylanthaquinone</td>
<td>Streptomyces sp.</td>
</tr>
<tr>
<td>Arenicolides</td>
<td>Salinispora arenicola</td>
</tr>
<tr>
<td>Aureolic acid</td>
<td>Streptomyces sp.</td>
</tr>
<tr>
<td>Aureoverticillactam</td>
<td>Streptomyces aureoverticillatus</td>
</tr>
<tr>
<td>Butenolides</td>
<td>Streptoverticillium luteoverticillatum</td>
</tr>
<tr>
<td>Chalcomycin</td>
<td>Streptomyces sp.</td>
</tr>
<tr>
<td>Daryamides</td>
<td>Streptomyces sp.</td>
</tr>
<tr>
<td>Elaiomycins B and C</td>
<td>Streptomyces sp.</td>
</tr>
<tr>
<td>Glyciapyrroles</td>
<td>Streptomyces sp.</td>
</tr>
</tbody>
</table>
Compounds and their sources:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitomycin C</td>
<td><em>Streptomyces lavendulae</em></td>
</tr>
<tr>
<td>Piericidins</td>
<td><em>Streptomyces sp.</em></td>
</tr>
<tr>
<td>Staurosporinone</td>
<td><em>Streptomyces sp.</em></td>
</tr>
<tr>
<td>Streptokordin</td>
<td><em>Streptomyces sp.</em></td>
</tr>
</tbody>
</table>

### Biological activity: anti-inflammatory

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclomarins</td>
<td><em>Streptomyces sp.</em></td>
</tr>
<tr>
<td></td>
<td><em>Salinispora</em></td>
</tr>
<tr>
<td></td>
<td><em>Arenicola</em></td>
</tr>
<tr>
<td>Salinamides A</td>
<td><em>Streptomyces sp.</em></td>
</tr>
<tr>
<td>and B</td>
<td></td>
</tr>
</tbody>
</table>

### Biological activity: antioxidant

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermacozines A</td>
<td><em>Dermacoccus</em></td>
</tr>
<tr>
<td>G</td>
<td></td>
</tr>
<tr>
<td>Actinosporins C</td>
<td><em>Actinokineospora sp.</em></td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
</tbody>
</table>

### Biological activity: antimalarial

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trioxacarcin</td>
<td><em>Streptomyces ochraceus</em></td>
</tr>
<tr>
<td>A, B, and C</td>
<td><em>Streptomyces bottropensis</em></td>
</tr>
</tbody>
</table>

The listed compounds and the strains were obtained from the report of Subramani and Aalbersberg, (2012) [36].

**Table 3.** Novel therapeutic substances isolated form halophilic actinomycetes

![Chemical structures](image-url)

**Figure 4.** Chemical structure of some therapeutic compounds produced by halophilic actinomycetes [5].
11. Biodegradable chemicals

Saline environments, particularly waters and soils, are always exposed to contamination by heavy metals or other toxic chemical substances due to anthropogenic activities [43]. The degradation of different substances in the environment is a continuous process due to the continued activities of microorganisms. Microorganisms degrade toxic compounds to nontoxic materials such as H₂O, CO₂, or other inorganic compounds [44]. Halophiles play a significant role in the biodegradation of the materials that contaminate marine environments. Actinomycetes have been reported to be one of those microorganisms that contribute in the degradation of organic compounds in the nature and play a role in the mineralization of organic matter [36]. Petroleum substances constitute one of the materials that contaminate marine habitats [45]. In a study carried out on oil-utilizing halophilic bacteria, results of phylogenetic studies showed that 30% of all isolates belonged to actinomycetes [46]. Extremely halophilic actinomycetes, *Streptomyces albiaxialis*, were reported to be able to grow in extreme environment by using crude oil as the sole carbon source in a salinity level up to 10% NaCl. Halophilic actinomycetes isolated from the oil-polluted soil in Russia were identified as *Rhodococcus erythropolis*. It was grown in a medium containing crude oil as a unique source of carbon. Results showed degradation of n-alkanes and iso-alkanes with chain lengths of C₁₁–C₃₀ and C₁₄–C₁₈ [47]. A new extremely halophilic actinomycete strain that belongs to *Actinopolyspora* sp. was isolated from saline and arid surroundings of an oil field in the Sultanate of Oman. This strain exhibited the capability of degrading alkanes up to C₁₅ and, at a slower rate, up to C₂₅ [48].

12. Industrially important enzymes

The extracellular enzymes produced by halophilic actinomycetes have significant advantages in biotechnological applications, such as biosynthetic processes, environmental bioremediation, and food processing. Most of the enzymes are active and stable at high temperatures and pH values and have ionic strength in the presence of organic solvents [49]. They have the capability of secreting extracellular enzymes such as lipase, DNase, protease, esterase, pullulanase, galactosidase, nuclease, xylanase, inulinase, cellulase, pectinase, gelatinase, alpha-glucosidase, beta-glucosidase, alpha-mannosidase, beta-mannosidase, chitinases, xylose isomerases, fructokinases, ribokinases, etc., which exhibit higher activity in alkaline pH and stability in high concentrations of organic solvents in their environment which have been reported in the last few years [50–52]. Among the industrially important enzymes, alkaline proteases and cellulase are produced by a number of microorganisms, but limited research was done on bulk production of alkaline proteases and cellulases by alkaliphilic actinomycetes. Cellulases and proteases are the most wanted extracellular enzymes and constitute 60% of global enzymes sales [53]. Reports claimed that a certain number of alkaliophilic and halophilic actinomycetes produce extracellular protease enzymes [54]. The halophilic protease secreted by the *Nocardiopsis prasina* HA-4 withstands a wide range of pH (7–10) and temperature (20–42°C). *Nocardiopsis halotolerans* and *Saccharomonospora halophila*
recovered from Kuwait showed better keratinolytic activity under high salt concentration [55]. *Streptomyces psammoticus* that secreted lignin degrading enzymes under alkaline conditions confirmed that the enzymes have applications in delignification of pulp, textile dye decolorization, and effluent treatment [53]. Cellulases are mainly involved in the production of second-generation bioethanol and textile processing industry [56]. Halophilic cellulases obtained from actinomycetes and from metagenomic library of some marine bacteria were reported to be halostable, thermostable, and alkalostable, all favorable for textile and laundry industries [57].

13. Advantage of using halophilic actinomycetes in the industry

Halophilic actinomycetes were suitable candidates for the expression of soluble recombinant proteins [58]. A broad range of plasmid vectors obtained from halophilic actinomycetes are used for cloning native promoters and heterologous promoters for the stable expression of gene for the production of the industrially important, compatible solute ectoine [59]. Since halophilic actinomycetes have antimicrobial activity and other extracellular enzymes production capability, there is less chance of contamination during fermentation. Therefore, the major cost for the sterilization of the cultivation media is reduced.

14. Conclusions and future prospects

Several halophilic actinomycetes have special biological and chemical defense systems such as pH tolerance, and stress-tolerant metals present one of the main sources for the discovery of novel metabolites, biosurfactants, several other chemicals, and commercially important enzymes with various applications. Despite the huge demand of synthetic molecules with effective antimicrobial properties, novel methods and technologies for discovering novel natural products from microbial sources from halophilic regions should be studied. The antimicrobial metabolites producing novel actinomycetes are good bugs for unsterile cultivation and continuous bioprocessing using seawater and low-cost-contributing media components. It will be interesting to identify the mechanism of the stable properties of halophilic enzymes, which may lead to significant novel biotechnological applications. Additionally, the stability of recombinant vectors in harsh conditions such as high salt, multiple metal ions, or organic solvents should be investigated. In conclusion, halophilic actinomycetes will be a useful host for the production of commodity chemicals, antibiotics, enzymes, and biofuels in bulk with low cost.

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