Joachim Wink
Fatemeh Mohammadipanah
Javad Hamedi *Editors*

Biology and Biotechnology of Actinobacteria

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Editors
Joachim Wink
Microbial Strain Collection;
Helmholtz-Centre for Infection Research
Braunschweig
Germany

Javad Hamedi
College of Science
University of Tehran
Tehran
Iran

Fatemeh Mohammadipanah
College of Science
University of Tehran
Tehran
Iran

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Preface

Actinobacteria are Gram positive organisms characterized by normally having a high mol% G+C ratio, filamentous or non-filamentous morphologies, and some members that produce spores through a differentiated developmental life cycle. The Class Actinobacteria comprises 6 classes, 6 orders, 14 suborders, and 56 families. The number of introduced genera of this taxon and their physiological diversity proposes that taxonomical identification of this group will be a dynamic process in future. Actinobacteria are autochthonous inhabitants of soil and marine and often among the dominant population of their ecosystems and may occur in extreme environments.

Members of the class Actinobacteria and the development of mankind are inseparably connected. The knowledge of this bacterial group goes back to the year 1839 when Corda identified *Actinomyces bovis* as the organism being responsible for bone swellings in bovine and to the year 1882 when Robert Koch identified *Mycobacterium tuberculosis* as the pathogen being responsible for tuberculosis. Since the 1940s, the history of antibiotic discovery and development is inseparably connected to Actinobacteria and especially to members of the genus Streptomyces. Streptomycin was the first antibiotic on market produced by an Actinobacterium and was developed in Waksman's laboratories. Today, we know that Actinobacteria exhibit large genomes (often more than 8 MB), which is one of the reasons for their potency to produce so many different bioactive metabolites. Actinobacteria of diverse genera like *Streptomyces, Saccharopolyspora, Amycolatopsis, Micromonospora,* and *Actinoplanes* are the producers of clinically used antibiotics and many other compounds that are used in pharmacy, agriculture, veterinary, animal husbandry, industry, and many other biotechnological fields.

Many of Actinobacteria are soil bacteria, but also pathogenic or saprophytic organisms belong to this group. Many of them show a characteristic differentiation by forming conidia that are usually regarded as spores. The conidia are arranged in spore chains, sporangia, or are found as single spores on sporophores. This high variation in their morphological form made taxonomy very difficult from the early beginning on. The cell differentiation we find in Actinobacteria is unique in microbiology and has been considered as model organisms to study the differentiation in Prokaryotes.

Actinobacteria are the most promising source of small bioactive molecules, and it is estimated that only 10% of actinobacterial bioactive chemicals have been

discovered to date. In this regard, the number and diversity of biosynthetic gene clusters in their genome justify their bioprospecting for new biological discovery. Actinobacterial sources estimate for about 45% of all microbial bioactive metabolites with 7,600 of these compounds (80%) being produced by the *Streptomyces* species. With more than 600 validly described species, *Streptomyces* is the largest genus in the bacterial world. Many of them have been isolated all over the world during industrial screening programs for many different purposes. However, rare Actinobacteria are also ubiquitous. Predominant and rare Actinobacteria surrounding us present in various ecosystems as free living, symbionts, or pathogens. They live in various sol, freshwater, seas and oceans, on surfaces of plants and animals, and into their cells or their cavities. Some actinobacteria can live in extreme environments, such as hot deserts, polar sites, acidic or alkaline soil and water or deep sea, and many other unusual habitats.

Despite the large number of studies on physiology, genetics, ecology, and applications of Actinobacteria, no up-to-date and comprehensive reference book covering all the mentioned aspects has been published until now. This book will see the emergence of insights about growth life cycle, cellular components, taxonomy, genetics, physiology, metabolism, and biotechnological applications to the name of fascinating aspects of Actinobacterial biology. We hope this book will contribute to a true understanding of all biological aspects of Actinobacteria.

Braunschweig, Germany — Joachim Wink
Tehran, Iran — Fatemeh Mohammadipanah
Tehran, Iran — Javad Hamedi

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Introduction

1

Javad Hamedi and Joachim Wink

Actinobacteria, as one of the largest bacterial phyla, is a dominant group of microorganisms being widely distributed in various terrestrial and aquatic ecosystems. These Gram-positive, high G+C content bacteria have received much attention in terms of studying its biology, majorly as they have been found to be tremendously potent in producing medically and industrially relevant secondary metabolites. This extensive secondary metabolism has led to the discovery of more than 120 antibiotics, different enzymes, enzyme inhibitors, and many other useful products from actinobacterial sources, discussion on which is one of the main focuses of the current book.

Apart from being designated as a controversial kind of microorganism when first discovered, namely, prokaryotic equivalent of fungi, the primary motivator for increasing the interest for biological basic studies on actinobacteria was the discovery and observation of their vast biotechnologically related potentials. That is to say, although being popularly accepted as a basic science, microbiology generally implements a bottom-up approach in studying microorganisms (including actinobacteria), and this seems to be an inherent property of the field. This is while other well-developed basic sciences such as *botany* or *zoology* have been basically formed on the basis of a top-down approach in which the objects being studied are considered, regardless to their importance in terms of application and consequently their potentials will be revealed after gaining an evenly developed knowledge.

The mentioned approach of microbiology consequently has resulted in an unbalanced and uneven development of basic biological knowledge for microorganisms,

J. Hamedi (✉)
Department of Microbial Biotechnology, School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran

Microbial Technology and Products Research Center, University of Tehran, Tehran, Iran
e-mail: jhamedi@ut.ac.ir

J. Wink
Helmholtz Center for Infection Research, Braunschweig, Germany

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having more basic information on those showing more application potencies. This pattern is also true in case of actinobacteria.

Accordingly, the understanding of the actinobacterial biology has been based on merely a few model organisms which are either medically important pathogens such as *Mycobacteria* or biotechnologically relevant producers including mainly *Streptomyces*. This is while there are many other actinobacterial groups with insignificant basic biological knowledge of which *Coriobacteriaceae*, *Catenulisporales*, the class *Thermoleophilia*, as well as many other taxa within this group can be named. However, it is also important to note that the yet-existing limitations in turning uncultured actinobacteria to cultivable ones are another shortcoming in understanding these bacteria which must be take into account. Yet the first steps to better and fully understand these bacteria to uncover their further potentials owe to addressing the problem of unevenly distributed basic biology of actinobacterial members.

Even though the taxonomic characterization has a very long tradition starting with its morphological features, today there are many open questions which also cannot be easily solved by sequence analysis of the organisms. Currently, many groups have their focus on the molecular characterization and the expressed features are often neglected. As the taxonomic description of novel actinobacteria can only be done by the use of a polyphasic approach, one aim of the book is to give an overview on the methods which can be used in the laboratory, even the more classic ones.

The search for novel antibiotics came into focus again in the last years because of the resistance development of many bacteria especially the nosocomial ones and the upcoming role of the so-called neglected diseases. The role of actinobacteria in this search for the future is still open, but the editors think that this class of bacteria is still one of the most promising groups even in the future. Therefore, we need more understanding of the biological role, the environment, and the bacterial communities for the isolation and identification of potential metabolite producers in the nature. Also the presentation of a straightforward identification procedure of interesting isolates is very important.

In this regard, the chapters of the present book are intended to provide a comprehensive view on the currently available issues relating to the biology and biotechnology of actinobacteria to hopefully depict and introduce the correct path for the development of a significant balanced understanding of the biology of this bacterial group.

Topics reviewed include all aspects of actinobacteria biology from their cellular properties, physiology, taxonomy, and genetics to their ecology and symbiosis. Despite these, their most important biotechnological traits are reviewed in the closing chapters to better elucidate the targeted trajectory which must be acquired to comprehensively cover the knowledge on actinobacteria as one of the most important bacterial phyla in medicine, industry, environment, and energy disciplines. As the final chapter of this book, the role of rapidly progressing omics data analysis and computational tools in the study of actinobacteria is also reviewed at a glance to not only stay synchronized with the novel paradigms of the post-genomic era of biology

but also to introduce these tools as efficient implementations to address the mentioned problem of unbalanced knowledge of actinobacteria in a time- and cost-effective manner.

Hopefully, the book will be of particular value to basic microbiologists and biotechnologists to unravel the great world of actinobacteria as well as bioinformaticians and molecular biologist who are trying to exploit biological data to find and address the existing biological problems with the aid of, namely, post-genomics approaches.

The Cellular Structure of Actinobacteria

2

Javad Hamedi and Naghmeh Poorinmohammad

2.1 Diversity of Cell Morphology in Actinobacteria

Encompassing more than 200 genera, actinobacterial members display remarkable variation in terms of cell morphology ranging from cocci in *Micrococcus* (Fig. 2.1a), rods in *Mycobacterium* (Fig. 2.1b), branched hyphae bearing spores such as *Micromonospora* (Fig. 2.1c), and mycelia that fragment into coccid and rod-shaped cells in *Nocardia* (Fig. 2.1d) to those actinobacteria which are produced from branched aerial hyphae of 0.5–2 μm in diameter like *Streptomyces* (Fig. 2.1e) (Qinyuan Li et al. 2016). It should be noted that in most cases, the actinobacterial life cycle presents different morphologies such as in *Arthrobacter* sp. which is depicted in Fig. 2.2.

Furthermore, depending on the culture condition, the cell morphology can differ. Accordingly, *Acidothermaceae* grows as slender filaments when the carbon source is glucose or cellobiose, and it grows as short rods when the carbon source is cellulose or xylan (Rosenberg et al. 2014).

Actinobacteria are currently classified and characterized using a polyphasic approach in which the microscopic morphological characteristics are of important indices. However, morphological profile cannot be often "exclusively" attributed to a specific actinobacterial taxon due to the high variation (Girard et al. 2013). For instance, members of the family *Actinomycetaceae* generally show properties such as Gram-stain-positive, straight or slightly curved rods some of which tend to form

J. Hamedi (✉) • N. Poorinmohammad
Department of Microbial Biotechnology, School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran

Microbial Technology and Products Research Center, University of Tehran, Tehran, Iran
e-mail: jhamedi@ut.ac.ir

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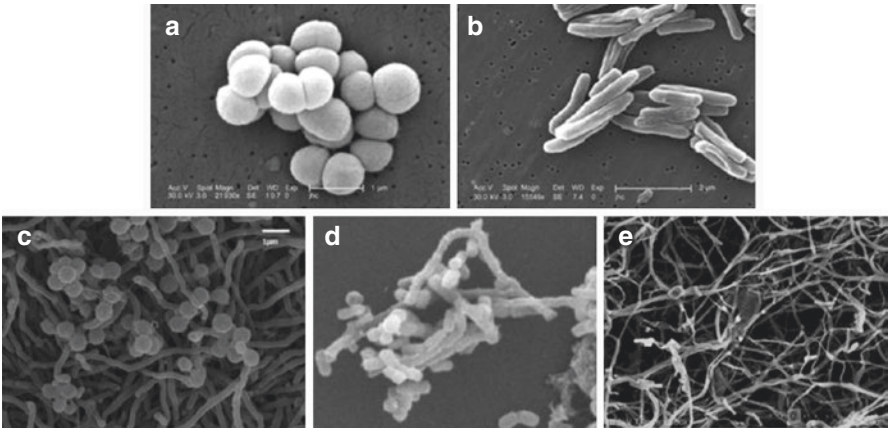

Fig. 2.1 Scanning electron microscope (SEM) images of (**a**) cocci of *Micrococcus luteus* (from Centers for Disease Control and Prevention (CDC), Public Health Image Library (PHIL), ID#:9761), (**b**) rods of *Mycobacterium tuberculosis* (from CDC, PHIL, ID#:9997), (**c**) branched hyphae of *Micromonospora schwarzwaldensis* (Gurovic et al. 2013), (**d**) fragmenting mycelia of *Nocardia asteroides* (Ribeiro et al. 2008), and (**e**) branched aerial hyphae of *Streptomyces mangrovisoli* (Ser et al. 2015)

branched filaments in which fragmentation occurs and rod-shaped or coccoid forms appear. However, the genus *Mobiluncus* (Fig. 2.3a) within this family exclusively exhibits curved, non-branching rods with which stains Gram-variable to Gram-negative although they possess a multilayered Gram-positive cell wall without an outer membrane (Hoyles and McCartney 2012). Some others form pleomorphic rods that occur singly or in many-celled chains or clumps; thus, they are not filamentous or spore forming such as the family *Bifidobacteriaceae* (Fig. 2.3b) (Rosenberg et al. 2014).

Table 2.1 summarizes the distribution of overall cell shape observed in the most-studied actinobacterial families.

2.1.1 Mycelium

Mycelial actinobacteria, e.g., members of *Streptomyces* genus, have a complex life cycle, which is considered in Chap. 3 in detail. Generally, there are two types of mycelia being developed by actinomycetes: substrate and aerial mycelia. Substrate or vegetative mycelium developed from the outgrowth of a germinating spore which grows on the surface of the culture medium to absorb nutrients. Some of these structures can produce pigments responsible for the colors of the substrate and aerial mycelia which

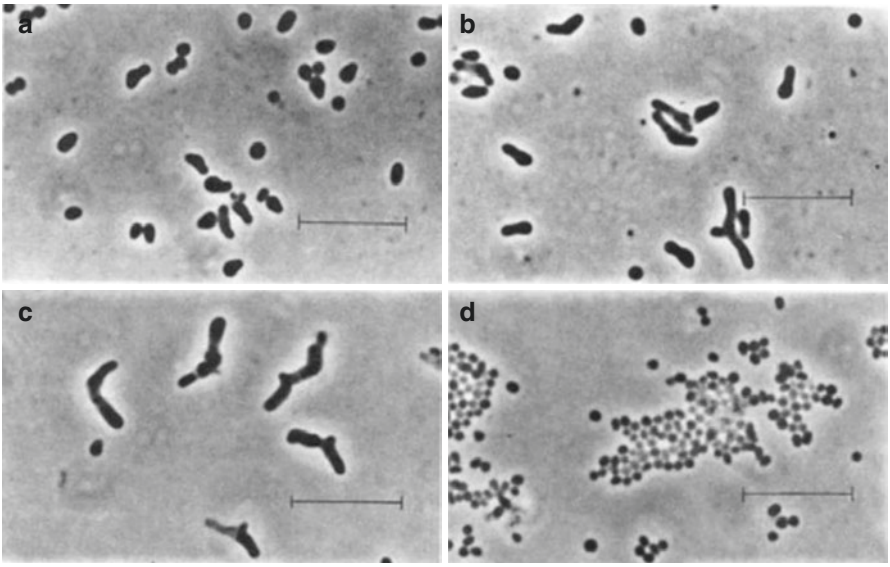

Fig. 2.2 Different morphological traits of *Arthrobacter globiformis* (**a**) after 6 h (rod to cocci), (**b**) after 12 h, (**c**) after 24 h, and (**d**) after 3 days (cocci). Bars = 10 μm (from Jones and Keddie 2006)

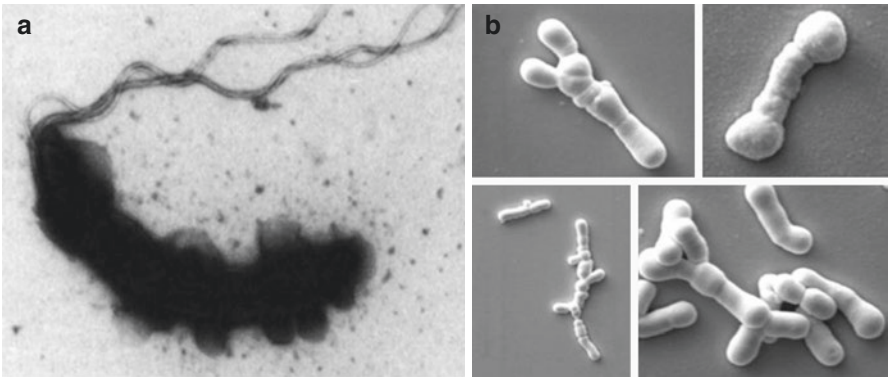

Fig. 2.3 Specific morphology of some actinobacteria (**a**) curve rods of *Mobiluncus curtisii* subsp. *curtisii* BV345-16 (here the flagella is also shown) (from Spiegel and Roberts 1984) and (**b**) different micrographs of pleomorphic bifidobacteria isolates (from Awasti et al. 2016)

also can be used for the identification of actinobacteria. Aerial hyphae, as the name denotes, grow into the air when the vegetative mycelium develops to a certain stage and will develop a reproductive hyphae producing spores. The aerial hyphae are enclosed in a fine sheath which does not exist in substrate mycelia (Ensign 1978).

Table 2.1 The distribution of cell morphology among actinobacterial families (Data are collected from the books "Bergey's Manual of Systematic Bacteriology" and "The Prokaryotes" as well as the web site of "List of prokaryotic names with standing in nomenclature (LSPN)" through www.bacterio.net, accessed August 2016)

Actinobacterial members		Cell shape			
Family	Number of genera within the family	Rod	Cocci	Rod-cocci	Filamentous
Acidimicrobiaceae	5	*		*	*
Acidothermaceae	1	*			*
Actinomycetaceae	8	*	*	*	*
Actinospicaceae	1				*
Beutenbergiaceae	4	*	*		
Bifidobacteriaceae	7	*			
Bogoriellaceae	3			*	
Brevibacteriaceae	1	*	*		
Catenulisporaceae	1	*	*		*
Cellulomonadaceae	6	*		*	*
Conexibacteraceae	1	*			
Coriobacteriaceae	14	*		*	
Corynebacteriaceae	4	*			
Cryptosporangiaceae	2				*
Demequinaceae	2	*			
Dermabacteraceae	4			*	
Dermacoccaceae	11			*	
Dermatophilaceae	5		*	*	
Dietziaceae	1	*	*	*	
Euzebyaceae	1	*			
Frankiaceae	2				*
Gaiellaceae	1	*			
Geodermatophilaceae	3				*
Glycomycetaceae	3				*
Iamiaceae	2	*		*	*
Intrasporangiaceae	23	*	*		*
Jiangellaceae	2				*
Jonesiaceae	1		*		
Kineosporiaceae	5	*	*		*
Microbacteriaceae	42	*	*		*
Micrococcaceae	15	*		*	
Micromonosporaceae	32				*
Mycobacteriaceae	2	*			
Nakamurellaceae	3		*	*	
Nitriliruptoraceae	1		*		
Nocardiaceae	8	*	*	*	*
Nocardioidaceae	9	*	*		*

Table 2.1 (continued)

Actinobacterial members		Cell shape			
Family	Number of genera within the family	Rod	Cocci	Rod-cocci	Filamentous
Nocardiopsaceae	8				*
Patulibacteraceae	1	*			
Promicromonosporaceae	7	*	*	*	*
Propionibacteriaceae	17	*	*		
Pseudonocardiaceae	31				*
Rarobacteraceae	1	*			
Ruaniaceae	2		*	*	
Rubrobacteraceae	1	*	*		
Sanguibacteraceae	1	*			
Segniliparaceae	1		*		
Solirubrobacteraceae	1	*			
Sporichthyaceae	1				*
Streptomycetaceae	10				*
Streptosporangiaceae	13				*
Thermoleophilaceae	1	*			
Thermomonosporaceae	6				*
Tsukamurellaceae	1	*		*	

Actinobacteria form a substrate mycelium in both submerged and solid-grown cultures, while aerial hyphae are differentiated specifically on solid surfaces. There are also exceptions in terms of mycelium formation. For instance, *Sporichthya* sp. produces short chains of aerial mycelium dividing into motile spores on an agar medium held by the holdfast, but no substrate mycelium formation is observed. The family *Micromonosporaceae* is also identified to develop extensive substrate mycelium while producing rudimentary aerial hyphae, or in some cases, no aerial mycelium is developed. Other actinobacterial taxa with absent aerial mycelium include some *Mycobacteria*, *Kineosporia*, and *Rhodococci* as well as the genus *Intrasporangium* and *Tsukamurella spumae* (O'Leary 1989; Rosenberg et al. 2014).

It is important to note that the morphological differentiation of actinobacteria, especially those with more extensive morphological differentiation such as *Streptomyces*, is tightly regulated and controlled by a truly organized mechanism through relevant genes. For more details regarding the regulation and morphogenetics, see Chap. 3.

2.1.2 Resistant Form of the Cells

Bacterial cells possess various differentiation states by which they form resistant non-vegetative cells as survival strategies under environmental challenges and unfavorable conditions. Accordingly, different resistant forms of bacterial cells are

observed such as endospores mainly in Bacilli and Clostridia, exospores which are as durable as endospores but form outside by growing or budding out from one end of the cell, cysts of *Azotobacter*, resting forms in the non-spore-forming *Arthrobacter*, and conidia in actinobacteria. Endospores are extremely resistant to heat (>100°C), many chemicals (i.e., acids, bases, alcohol, chloroform), desiccation, and radiation due to the spore's inherent properties such as high concentration of specific DNA-protecting proteins as well as the dehydration of the cytoplasm and impermeability of the endospore coat. *Azotobacter* species, the diazotroph Gram-negative Proteobacteria famous for their biopolymer production in biotechnology, form cysts which, unlike endospores, can only resist desiccation and some chemicals but not the high temperature. The genus *Arthrobacter* forms cyst-like resting cells with extremely lowered metabolism under unfavorable environments such as in response to conditions of severe carbon and energy deficiency.

The actinobacterial "conidia," which are commonly called spores, are part of the reproductive process, although they are also capable of surviving for long periods of time. Thus, conidia are involved in both replication and survival, while cysts and spores are merely for survival strategies. Here, we use the more commonly used phrase of "spores" instead of the correct term "conidia" for actinobacterial-resistant form of cell.

Classic actinomycetes form well-developed substrate mycelia and develop branched aerial hyphae on the vegetative mycelia and can produce spores directly or in the form of sack-like ultrastructures.

The ability of forming such complicated structures (spores, sporangia, and sporangiospore) as well as the pattern of substrate mycelium fracture, the number of spores and their position, the shape of sporangia, and whether the sporangiospore has flagella or not are used in the classification of some actinobacteria.

These spores can be organized as single in *Saccharomonospora* (Fig. 2.4a), bispores in *Microbispora* (Fig. 2.4b), or spiral chains of multiple spores such as in *Streptomyces* (Fig. 2.4c). However, in some genera such as *Nocardia*, *Mycobacterium*, and *Rhodococcus*, the developed mycelia fragment into coccoid or rod-shaped elements.

In general, actinobacterial spores are formed by hyphal origin which is further classified into three subtypes, namely, arthrospores (subdivision of sheathed hypha) as in *Streptomyces*, *Actinopolyspora*, *Nocardia*, etc.; aleuriospore (subdivision of sheathless hypha) in *Micromonospora*, *Saccharopolyspora*, etc.; and fragmentation spores of *Rhodococcus*, *Nocardiopsis*, etc. as mentioned before. Some other actinobacteria especially *Frankia* species have special vesicles called sporangia containing whether aplanospores (non-motile) or planospores (motile). Planospores or motile spores can possess a single flagellum per spore (monotrichous) or appear with multiple flagella with different arrangements around the cell. Planospores can be seen in *Sporichthya*, *Ampullariella*, *Spirillospora*, and *Catenuloplanes*. These motile spores possess a kind of smooth surface, while aplanospores can have

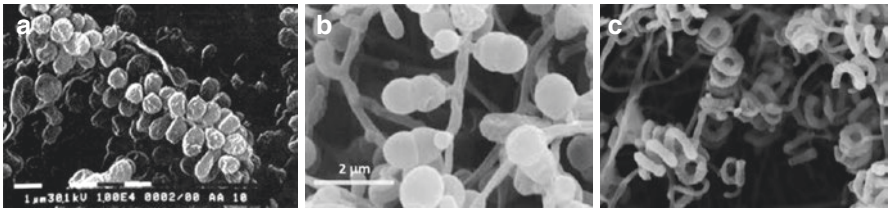

Fig. 2.4 Different spore organization in actinobacteria. (**a**) *Saccharomonospora glauca* (Greiner-Mai et al. 1988), (**b**) *Microbispora corallina* (Nakajima et al. 1999), and (**c**) *Streptomyces* sp. MN 2 (Kumar et al. 2014)

different surface characteristics whether smooth or rough with warty, hairy, spiny, or rugose ornamentations. Aplanospores are also produced by *Micromonospora*, *Streptomyces*, *Actinomadura*, and *Microtetraspora* (Locci 2006).

It must be noted that in some individual genus or even species, two kinds of spores can be formed such as in *Nocardia* which is able to produce both fragmentation spores and arthrospores. On the other hand, spores do not form exclusively on aerial hyphae, and the formation of spores may also originate from the vegetative mycelium that has been observed in *Actinobifida* and some members of *Streptomyces* (Kalakoutskii and Agre 1976).

Spores produced in the form of chains are 2 to more than 50 spores long depending on the genus and are thicker than the mycelium when they are arranged in short chains, while long spore chains are equal with the hyphae in diameter (Li et al. 2016). Long spore chains (up to 100 spores) have been observed in *Streptomyces*, *Nocardioides*, *Streptoverticillium*, and *Kitasatospora*, and the genera *Actinomadura*, *Sporicthya*, *Catellatospora*, *Saccharopolyspora*, and some *Nocardia* produce short chains of spores (Mayilraj et al. 2006; Taddei et al. 2006; Rosenberg et al. 2014). Long spore chains of *Streptomyces* differ in terms of spatial organization and are classified as rectiflexibile type (Fig. 2.5a), retinaculiaperti type (Fig. 2.5b), spira type (Fig. 2.5c), and verticillati type (Fig. 2.5d) (Qinyuan Li et al. 2016).

Generally, the shape of actinobacterial spores is often spherical, although other shapes may be observed such as cuboid in *Chainia*, oval in *Actinomadura*, or claviform spores of *Dactylosporangium*. Moreover, mature spores usually show a variety of colors such as white, pink, gray, blue, and so on.

Sporangia, the bag-like structures for the development and release of spores, also vary vastly on the basis of shape and size. They are formed whether on substrate or aerial mycelium and can be globose (Fig. 2.6a) (*Spirillospora*, *Streptosporangium*), cylindrical (Fig. 2.6b) (*Planomonospora*, *Planobispora*), claviform (Fig. 2.6c) (*Dactylosporangium*), and in other shapes while they are 2–50 µm with most of them being 10 µm in size (O'Leary 1989).

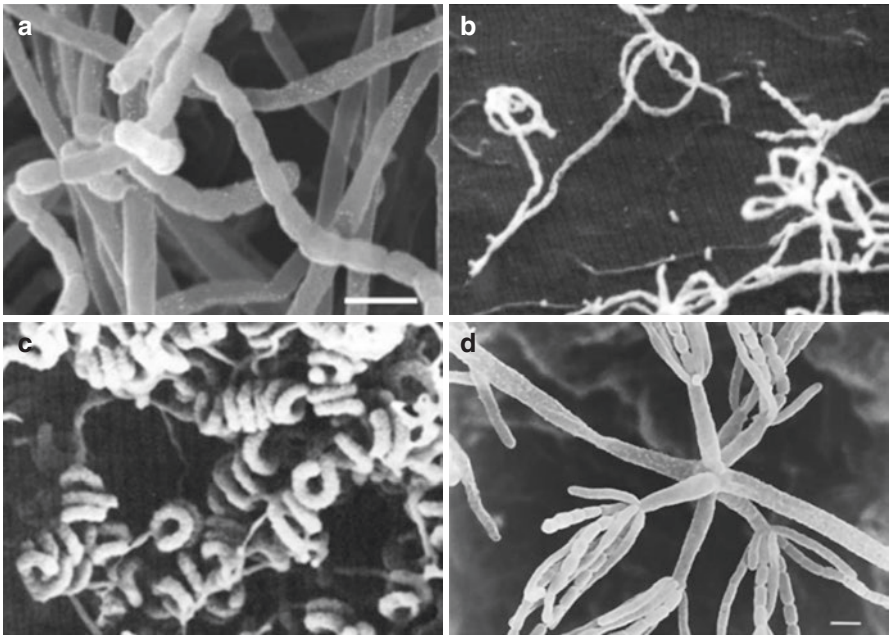

Fig. 2.5 Morphology of the long spore chains of *Streptomyces* species. (**a**) *Streptomyces plumbiresistens* (Guo et al. 2009), (**b**) *Streptomyces vinaceus* (Ludwig et al. 2012), (**c**) *Streptomyces hygroscopicus* (Ludwig et al. 2012), and (**d**) *Streptomyces verticillus* (Produced by Harada and Hamada)

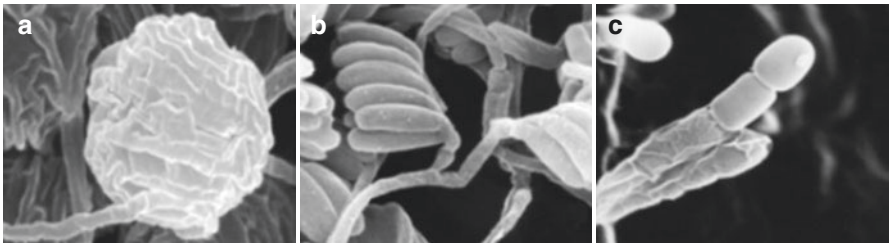

Fig. 2.6 Sporangia morphology in some actinobacteria. (**a**) *Spirillospora albida* (Produced by Vobis), (**b**) *Planomonospora parontospora* (Produced by Hayakawa, Iino, and Nonomura), and (**c**) *Dactylosporangium fulvum* (Produced by Shomura)

Just like spores, sporangia have different types based on the number of spores. Sporangia with few spores may be called oligosporous sporangia while polysporous ones contain numerous spores as the name denotes. Most of the actinobacterial members forming sporangium produce planospores although exceptions exist as for *Stretosporangium* and *Kutzneria*.

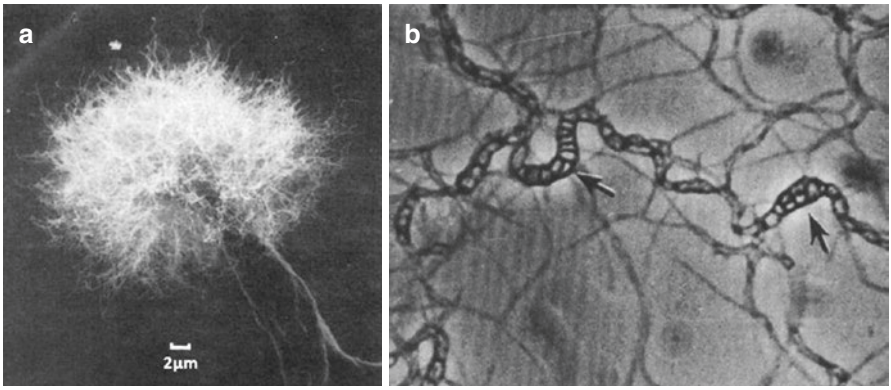

Fig. 2.7 Sporangia-like structures. (**a**) Synnemata in *Actinosynnema* (Hasegawa et al. 1978), (**b**) sclerotia (*arrows*) in *Chainia barodensis* (Ganju and Iyengar 1974)

Finally, there are other less-studied types of reproductive structures reported in actinobacteria such as columnar hyphal structures called synnemata (Fig. 2.7a) which bear chains of conidia in *Actinosynnema* (Land et al. 2009) and sclerotia (Fig. 2.7b) in some *Streptomyces* and *Chainia* (Subramanian 2016).

Generally, actinobacterial developmental life cycle is uniquely complex, especially in case of actinomycetes which form spores and mycelium. Their life cycle involves coordinated multicellular development with both physiological and morphological differentiation of several cell types as discussed. The life cycle of actinobacteria is specially studied for *Streptomyces*. This genus has been the subject of intense genetic and molecular biology research, while there are little or no information regarding the developmental process of many other actinobacterial members which is an issue to be addressed.

Typically, when a *Streptomyces* spore encounters appropriate conditions in terms of environmental and nutritional factors, it germinates and grows to form the hyphae which then grow by tip extension and further branches into the vegetative mycelium. When conditions become unfavorable, both production of secondary metabolites and morphological differentiation are initiated. In this condition, aerial hyphae break the surface tension and grow into the air and subsequently switch from extension to septation. The aerial hyphae become divided by a developmentally controlled form of cell division into long chains of prespore compartments, which then develop the mature spore (Fig. 2.8). It is important to note that the importance of studying the mentioned features especially in the identification of these bacteria is directedly discussed in Chap. 11.

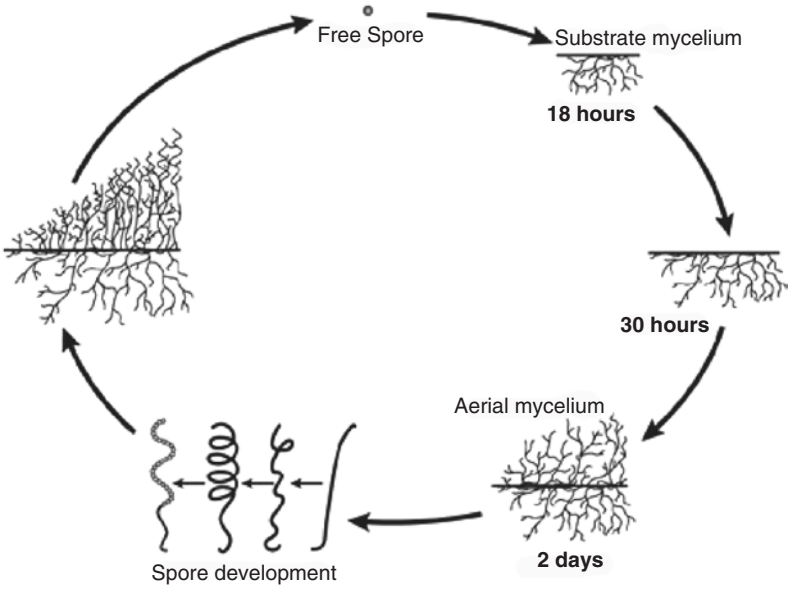

Fig. 2.8 *Streptomyces coelicolor* life cycle (From Angert 2005)

2.2 Cell Envelope

Bacterial cell envelope is acceptably defined as the membrane(s) and other structures that surround and protect the cytoplasm of the bacteria. This multilayered structure is quite complex and serves as an important element in the living and survival of the organism with sophisticated mechanisms (Silhavy et al. 2010).

Apart from determining cell shape and permeability, bacterial cell envelope serves as the interface for interacting with other bacteria, environment, and the host if it is pathogen. Thus, the study of this compartment is of great importance especially in medicine and biotechnologically relevant processes (Braun et al. 2015). Moreover, chemotaxonomy which is shown to be an effective strategy for the classification of actinobacteria is concerned with the distribution of specific chemicals of the actinobacteria cell envelope such as amino acid, sugar, polar lipids, menaquinones, and mycolic and fatty acids (Diagne et al. 2013).

The study of actinobacterial cell envelope is generally limited to the most important pathogenic members such as the distinctive family of *Corynebacteriaceae* including mainly *Mycobacterium*, *Rhodococcus*, and *Nocardia* as well as the biotechnological workhorses within the family like *Corynebacterium glutamicum*. Therefore, in this chapter, these organisms are mostly considered to as model organisms for the study of cell envelope (Fig. 2.11).

2.2.1 Plasma Membrane

The plasma membrane of the mentioned bacteria does not differ widely from other bacteria. In the case of this plasma membrane, an unusual lipid named diacyl phosphatidylinositol dimannoside (Fig. 2.8) is reported to be the dominant component of the inner leaflet in *Mycobacteria* which is proved to cause the general drug resistance in this genus due to its effect on the membrane fluidity which slows the influx of the drugs (Bansal-Mutalik and Nikaido 2014). In addition to the conventional plasma membrane which does not differ with other bacteria (comprising mainly phosphatidylglycerol), *Corynebacteriaceae* possess an outer layer of lipids surrounding the cell wall, which is different from the inner membrane, as in Gram-negative bacteria. This means that this unusual property among Gram-positive bacteria gives *Corynebacteriaceae* an extra outer permeability barrier. This outer membrane is an asymmetrical bilayer which is about two times thicker than the plasma membrane in *Corynebacteriaceae* (Brennan and Nikaido 1995), although *Corynebacteria* lack the periplasmic-like space which is present in *Mycobacteria.* The aforementioned thickness is reported to be due to phosphatidylinositol mannosides (PIMs) (Fig. 2.9) of the outer leaflet which are restricted to the members of actinomycetes (Brennan and Nikaido 1995).

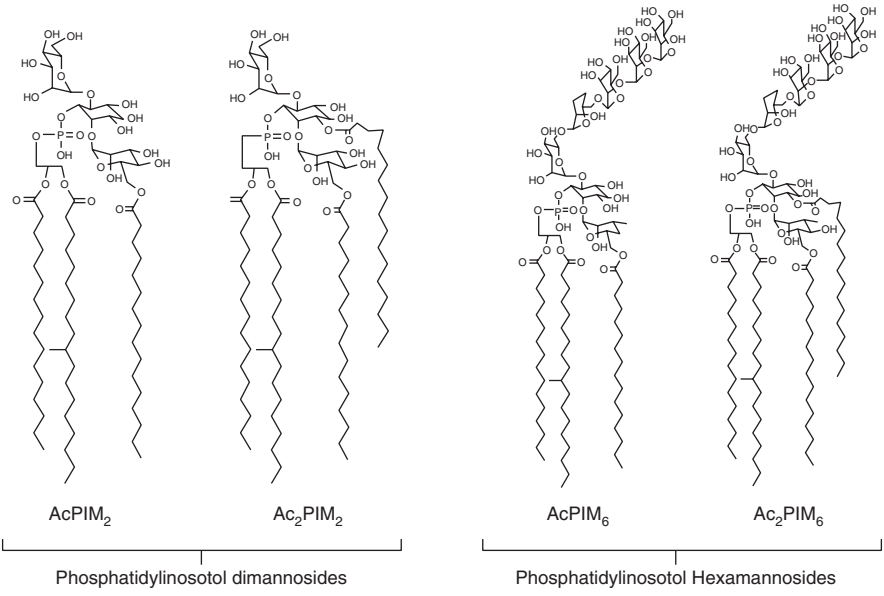

Fig. 2.9 Different types of PIMs. *$AcPIM_2$* monoacyl phosphatidylinositol dimannosides, *Ac_2PIM_2* diacyl phosphatidylinositol dimannosides, *$AcPIM_6$* monoacyl phosphatidylinositol hexamannosides, *Ac_2PIM_6* diacyl phosphatidylinositol hexamannosides

The inner layer of the asymmetrical outer membrane majorly consists of mycolic acid (2-alkyl branched, 3-hydroxy long-chain fatty acids) which is covalently attached to arabinogalactan units in the cell wall. The length of carbon chain in mycolic acid is shorter in *Corynebacteria* than that in *Mycobacteria*, being C_{22} to C_{36} and C_{60} to C_{90}, respectively (Schluesener et al. 2005). Rhodoccocal mycolic acids are of intermediate length typically with 28–54 carbons in total (Sutcliffe et al. 2010). The outer leaflet is composed of different anchored glycolipids, glycopeptidolipids, sulfolipids, and phospholipids. The mycobacterial phospholipids are mostly the derivatives of phosphatidic acid such as phosphatidylglycerol, phosphatidylethanolamine, cardiolipins, phosphatidylinositol, and PIMs (Brennan and Nikaido 1995). There are also some lipids found in small amounts showing no structural roles in the plasma membrane, but they rather seem to have functional effects as sugar donors, among which the two well-studied examples are polyprenol phosphomannose (PPM) and decaprenol phosphoarabinose (DPA). They are donors for mannose and arabinose, respectively (Crellin et al. 2013).

The polysaccharides of the outer membrane are primarily composed of a high-molecular-mass glucan and arabinomannans (Puech et al. 2001).

Since the outer membrane exerts a permeability barrier in this family, substance exchange, especially for water-soluble molecules, along the cell envelope is performed via many specific and nonspecific proteins located in the outer membrane. In *M. tuberculosis*, more than 140 of these proteins are predicted through genome mining, while many of them are still not fully characterized (Song et al. 2008). For instance, the nonspecific protein named MspA is the main porin responsible for the transport of glucose, metal ions, phosphate, and amino acids in *Mycobacteria* which possess an octameric 16-stranded β-barrel structure (Niederweis et al. 1999). The anion channel PorB of *Corynebacterium glutamicum* and the cation channel Rv1968 in *Rhodococcus jostii* are other examples of such porins (Sutcliffe et al. 2010). The elucidation of the structure of such porins in pathogenic actinobacteria will help in finding inhibitors as a drug target to fight against the bacterium; however, only few of them are characterized in terms of 3D structure. Additionally, there are other types of proteins in the outer membrane which are responsible for the transport of low-abundance solutes for which the porin pathway is not efficient (Niederweis et al. 2010). Interestingly, since *Mycobacteria* can use lipids as carbon source for growth, there are also protein channels found in the outer membrane such as Mce4 which enables cholesterol to enter the cell (Pandey and Sassetti 2008).

Other membrane-associated components are a few types of polyterpenes shown to be associated with the protection against photolytic damage. Mycobacterial carotenoid is one of these products which causes the yellowish orange color in *M. kansasii* (Brennan and Nikaido 1995). There are also respiratory isoprenoid quinones in the cytoplasmic and mitochondrial membrane found to date merely in actinobacteria. The number of isoprene units and hydrogenated double bonds in different actinobacteria is of considerable value in the chemotaxonomy of these bacteria (Rousseaux et al. 2001).

As another membrane component, hopanoids, the structurally and biosynthetically similar components to sterols of eukaryotes, can be reviewed for their existence and role in some actinobacteria. Hopanoids are structurally diverse; however, some of the most common structures are illustrated in Fig. 2.10.

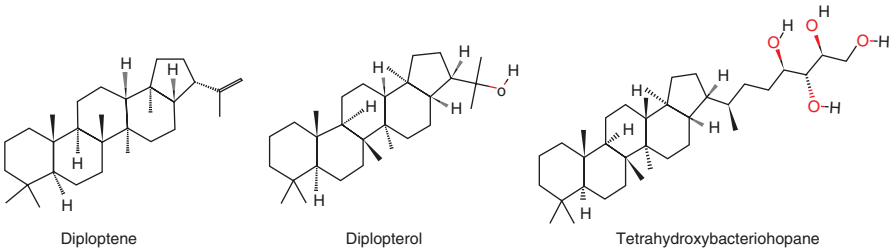

Fig. 2.10 Chemical structure of some common hopanoids

In general, hopanoids or bacteriohopanepolyols (BHPs) are a class of pentacyclic triterpenoids which are generally localized in the cytoplasm, outer membrane, and also in association with other specialized bacterial membranes such as the thylakoid membranes in cyanobacteria and in the vesicle envelope of the root nodule symbionts *Frankia* sp. (Welander et al. 2009; Sáenz et al. 2015). Eukaryotic sterols such as cholesterol are responsible for modulating the membrane order, and hopanoids are shown to perform the same role in bacteria except that the condensing and ordering effect of these components is elucidated to be weaker than that of cholesterol (Poger and Mark 2013), although it significantly contributed in the membrane functionality.

As hopanoid-containing actinobacteria are *Frankia* sp. and the nitrogen-fixing actinomycetes, these triterpenoids are produced in extremely high levels in these bacteria. Hopanoids are the most abundant lipids in the nitrogen-fixing vesicles of this genus (diazovesicles which will be discussed later in this chapter) and are believed to be efficient in protecting the nitrogenase against oxygen by acting as a major physical barrier as they represent up to 87% of the total vesicle lipids detected (Nalin et al. 2000). Hopanoids which are produced in nearly half of all analyzed bacterial species have proven to be physiologically important due to their critical role in membrane dynamics during different stresses. For instance, the membrane condensing hopanoids of *Streptomyces coelicolor* is proposed to have possible roles in stress alleviation in aerial mycelium by diminishing water permeability across the membrane (Poralla et al. 2000) although they are verified to be not essential for growth when the gene for hopanoid biosynthesis was mutated (Seipke and Loria 2009).

2.2.2 Cell Wall

Actinobacteria possess a cell wall containing peptidoglycan (PG) layer of 20–80 nm thickness. Twenty percent of the cell dry weight is represented by the cell wall of which around 60% is the PG weight. The remaining weight is due to the presence of different macromolecules such as lipids, polysaccharides, proteins, and teichoic acids in which some of them are free while others are attached to the PG.

Conventionally, bacterial PG is a polymer containing β1–4 linked *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) units as well as a chain of amino acids connected to NAM. In actinobacteria, variations in the amino acid sequence of these peptide chains together with their mode of cross-linkage give clues for their classification.

The amino acid of position 3 in the peptide chain is the most helpful index for the classification of actinobacteria based on cell envelope compositions. This position can be occupied with *meso*- and LL-diaminopimelic acid, L-ornithine, L-lysine, and L-diaminobutyric acid (Roy et al. 2007).

Apart from peptidoglycan, the cell wall of actinobacterial members also includes arabinogalactan (AG), lipomannan, and lipoarabinomannan. Many of the sugar polymers of the cell wall, such as AG, are important due to their role in immunological reactions of the host, when talking about pathogenic actinobacteria. There are several antibiotics targeting AG such as ethambutol, since the absence or defect in AG is verified to cause growth defects (Alderwick et al. 2006; Seidel et al. 2007).

The cell wall core of *Mycobacteria* is composed of PG covalently attached to AG via phosphoryl-Nacetylglucosaminosyl-rhamnosyl linkage units (PGlcNAc-Rha), while AG esterifies to long-chain mycolic acids which is the main component of the inner leaflet of the outer layer. This is while in other Gram-positive bacteria, wall teichoic acids (WTA) are covalently attached to PG (Grzegorzewicz et al. 2016).

Although lipoteichoic acid (LTA) is suggested to be available merely in Firmicutes, they are reported in two actinobacterial genera, namely, *Agromyces* and *Streptomyces* (Potekhina et al. 1982; Greiner-Mai et al. 1987), as well as in *Thermobifida fusca* which was suggested to have roles in the cell envelope homeostasis (Rahman et al. 2009).

Another major compartment of actinobacterial cell wall is teichuronic acids, the heteropolymeric polysaccharides composed of a uronic acid along with amino sugars and neutral monosaccharides which are linked to a polymer of whether amino acids or glycerol phosphates (Tul'skaya et al. 2011). They are of common cell wall components of actinobacteria such as *Propionibacterium*, *Corynebacterium*, *Catellatospora*, *Actinoplanes*, *Streptomyces*, and *Kribbella* (Tul'skaya et al. 2011).

2.2.3 Surface Layer and Capsule

Surface layer or S-layer is a crystalline monomolecular outermost layer of cell envelope composed of identical proteins or glycoproteins found in the cell envelope of various bacterial taxonomic groups as well as in some members of actinobacteria. Cells equipped with S-layer can efficiently withstand the osmotic pressure. Moreover, it is proposed that the existence of this layer which serves as the interface between the cell and its environment would give the functionality as a selective sieve to the cell by which the passage of low-molecular-weight solutes is allowed while excluding large molecules or structures (such as viruses) (Guerrero 2000).

C. glutamicum possesses an S-layer composed of PS2 proteins. PS2 is a 52 kDa protein which forms an S-layer with hexagonal lattice symmetry (Peyret et al. 1993). This layer has been shown to give the bacterium an extreme resistance to protease and detergents. The S-layer is attached to the cell wall majorly via tight hydrophobic interactions. The C-terminal segment of PS2 is hydrophobic, and any defect in this segment has been shown to result in the secretion of this protein to the medium. As a biotechnological point of view, since bacteria with S-layer express the relating protein in high amounts and within an efficient expression system, they can be biotechnologically studied and used for heterologous protein expression procedures (Bayan et al. 2003).

Rhodococcus equi which is a human and animal pathogen actinobacterium produces capsular polysaccharides which are structurally diverse acidic heteropolysaccharides consisting of acetal-linked pyruvate or lactic acid ether substituents. Other actinobacteria such as *Thermomonospora*, *Arthrobacter*, *Acidothermus*, and *Nitriliruptor* also produce capsules (Rosenberg et al. 2014). Although capsules are not observed by capsule staining, *Actinobaculum* is reported to have a fringelike outer coat external to the cell wall in thin section electron micrographs (Rosenberg et al. 2014).

Some pathogenic *Mycobacteria* also produce capsules as a thick layer in the outermost part of the cell. *M. tuberculosis* and *M. kansasii* possess glucan and protein-containing capsules, while in the case of *M. leprae*, capsule is comprised of phenolic glycolipids (Daffé 2015) (Fig. 2.11).

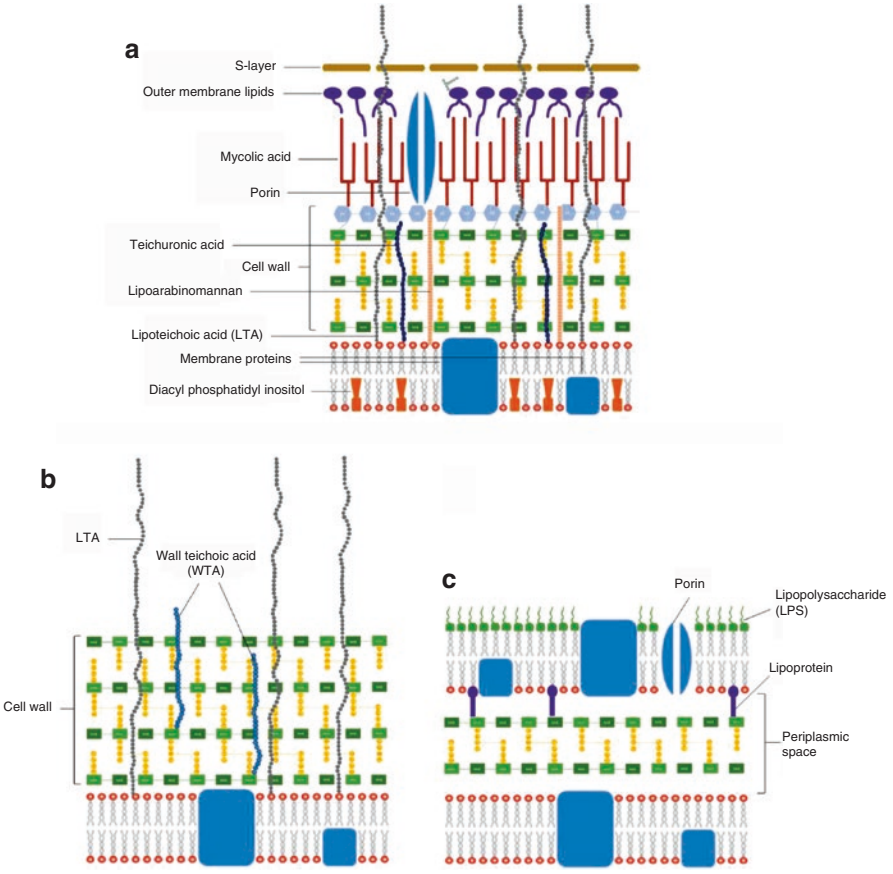

Fig. 2.11 The schematic cell envelope of *Corynebacteriaceae*. (**a**) Gram-positive (**b**) and Gram-negative cell envelope (**c**) are also depicted for quick comparison. *Notes:* (*1*) Dimensions are not fully considered; (*2*) Gram-positives and Gram-negatives may also contain S-layer; (*3*) Not all the depicted components in "a" are found altogether in every member of Corynebacteriacea (see text) and many of other actinobacteria, e.g., Micrococcus luteus has pattern "b"

2.3 Cytoplasm

2.3.1 Cytoskeleton

One of the major components of bacterial cytoplasm is the "cytoskeleton," which is required for cell shape determination, cell division, and motility (Graumann 2009). Cytoskeletal proteins are ubiquitous among bacteria such as FtsZ, MreB/Mbl, and crescentin which are the homologues of eukaryotic tubulin, actin, and intermediate filaments, respectively, as well as unique bacterial cytoskeletal proteins such as MinD and bactofilins.

Streptomycetes have an extremely elaborated cytoskeleton which is more complicated than other bacteria which can be explained by their hyphal growth (Celler et al. 2013). *Streptomycetes* possess besides FtsZ and MreB/Mbl a large number of proteins with coiled-coil structural elements. In these bacteria, as well as other Gram-positives, DivIVA exists but with divergent function: in *S. coelicolor*, DivIVA is essential for growth in and localizes to tips to drive apical growth. Here, the overexpression of Scy, a novel coiled-coil cytoskeleton protein which colocalizes with DivIVA, results in establishing growth nuclei for apical growth and branching by sequestering the DivIVA (Lin and Thanbichler 2013). The intermediate filament protein (FilP) localizes near the DivIVA/Scy at the hyphal tip which is proposed to have roles in mechanically controlling the cell shape (Fig. 2.12).

Moreover, actinobacteria possess actinobacterial-specific regulators of FtsZ which is essential in the initiation and development of cell division. FtsW is involved

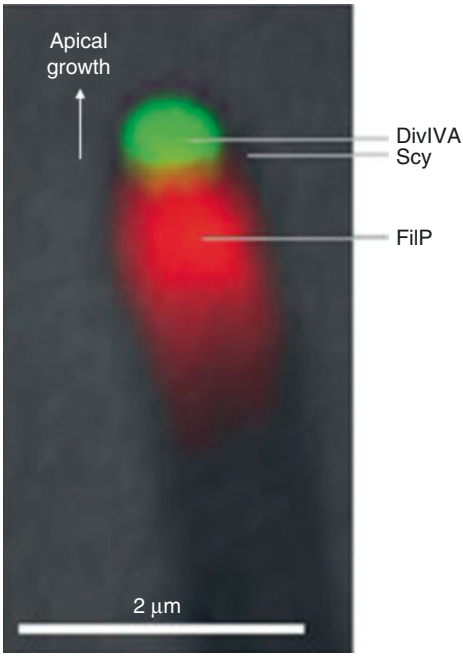

Fig. 2.12 Cytoskeleton proteins contributing in the apical growth of *Streptomyces coelicolor* (Adapted from Fuchino et al. 2013)

in the positive regulation of cell division in *Mycobacteria* which does not show any homology with its similar molecules within other bacterial genera. Another example is DivS which suppresses the cell division in *Corynebacteria*. The existence of these specific proteins lacking in other bacteria proves the complexity of the actinobacterial cell division and growth (Letek et al. 2012).

2.3.2 Inclusion Bodies

Intracytoplasmic storage compartments called inclusion bodies are widespread among bacteria as well as eukaryotes in which nutrients are accumulated. Lipophilic storage elements represent an efficient form of energy storage since lipids provide more energy than carbohydrates and proteins. This form is found mainly in eukaryotes, although there are few bacteria belonging to the actinobacteria able to store triacylglycerols such as *Mycobacterium* and *Nocardia*. The formation of polyhydroxyalkanoic acid (PHA) inclusions has been also reported in *Nocardia*, *Streptomyces*, *Kineosphaera*, and *Rhodococcus* species (Matias et al. 2009). It is important to note that the granules of PHAs can also be visualized inside the spores (Matias et al. 2009).

Other than PHA and triacylglycerol, other lipids can also be accumulated in actinobacteria. In this regard, *Nocardia* can accumulate glycerides and straight-chain waxes when grown on specific hydrocarbons (Alvarez et al. 1996).

Pallerla et al. have reported the presence of volutin granules, the intracellular storages of complexed inorganic polyphosphate, in *C. glutamicum* which is accumulated for about 18–37% of its total cell volume (Pallerla et al. 2005). The formation of polyphosphate (or metachromatic) granules was previously reported in *C. diphtheriae*, identification of which is performed via staining with toluidine blue or Neisser stains and was a diagnostic tool to discriminate the highly pathogenic *C. diphtheriae* with other corynebacteria (Mac Faddin 1985).

2.3.3 Bacterial Microcompartments (BMCs)

BMCs are organelles composed of merely proteins which were previously believed to be phages due to their polyhedral and polygonal shape. They organize diverse metabolisms by encapsulating the relevant enzymes of the metabolic process and are generally used to optimize pathways with toxic or volatile intermediates. When these compartments are lacking, the accumulation of such toxic compounds would result in cellular toxicity. On the other hand, they might diffuse from the membrane to the environment which is unfavorable due to carbon loss. There are different types of well-known BMCs such as carboxysome, 1,2-propanediol utilization (Pdu), and ethanolamine utilization (Eut) microcompartments which diffuse polar molecules such as CO_2, propionaldehyde, and acetaldehyde, respectively. The latter two compounds are cytotoxic, and these BMCs contain enzymes to produce nontoxic derivatives, while carboxysomes benefit in CO_2 fixation due to bringing high concentrations of relevant enzyme, substrate, and cofactors together (Chowdhury et al. 2014).

It is suggested that the genes encoding BMCs have been subjected to frequent horizontal gene transfer and are thus widespread among the bacterial phyla. BMC gene cluster has been found in many actinobacteria with their relevant enzyme. *Mycobacterium smegmatis* (aldehyde dehydrogenase), *Nakamurella multipartite* (aldehyde dehydrogenase, glutathione-dependent formaldehyde dehydrogenase), and *Solibacter usitatus* (aldehyde dehydrogenase, aldolase, dihydrodipicolinate synthase) are some of these examples (Kerfeld et al. 2010).

2.4 Other Cellular Structures

2.4.1 Vesicles

Gas vesicles are cytoplasmic gas-filled bubbles, whose function is to control the organism's floatation process in aqueous environments. They have been widely studied in cyanobacteria as well as halophilic archaea; they are also reported in actinobacteria distributed in soil and aqueous environments.

The envelope of these vesicles is consisted of a specific type of amphiphilic proteins, namely, gas vesicle proteins (Gvps), whose genes (*gvp*) can be mined in bacteria to predict the presence of gas vesicles. The occurrence of orthologues of the eight essential *gvp* genes has been reported in *Streptomyces* sp. which are verified to be in duplicate or even triplicate in the genomes of these bacteria. However, the actual synthesis of these vesicles should be experimentally validated. The alternative function of Gvps in these bacteria is suggested to be in the osmostress response (Shively 2006). Other actinobacterial members such as *Saccharopolyspora erythraea*, *Rhodococcus* sp., and *Amycolatopsis balhimycina* also contain a *gvp* gene cluster. However, the discovery of the "diazovesicles" in *Frankia* sp. is a better example in this regard. *Frankia* are filamentous, nitrogen-fixing actinomycetes which live as microsymbionts in many plant hosts. During nitrogen-limited aerobic conditions in culture and in several types of actinorhizal root nodules, *Frankia* develops stalked, spherical vesicles that develop from hyphal branches. These vesicles are covered with a lipid-abundant layer which mainly consists of hopanoids, and due to this, hopanoid level in *Frankia* is among the highest level among bacteria (Dobritsa et al. 2001). This lipid envelope forms a barrier to oxygen which in turn will protect the nitrogenase enzyme.

2.4.2 Flagella and Pili

As previously mentioned, actinobacterial taxa such as *Actinoplanes*, *Planomonospora*, *Dactylosporangium*, *Ampullariella*, *Spirillospora*, *Planobispora*, and *Dermatophilus* produce motile spores possessing flagella with different numbers and organizations. Although all types of flagellation are found in these bacteria, peritrichous flagellation is rather rare (Kalakoutskii and Agre 1976). Furthermore, flagellated spore released from old sporangia has been identified to be non-motile in *Actinoplanes* sp.; however, the motility can be restored if a suitable carbon and energy source is added to the solution. These bacteria can resynthesize flagella if kept in suitable medium with

sufficient amino acid and carbon sources when deflagellated via methods such as ultrasound treatment (Kalakoutskii and Agre 1976).

The flagella may appear around the spore through different organizations as can be seen in Fig. 2.13.

As a few examples, *Actinoplanes* sp. have globose motile spores with a polar tuft of flagella (Fig. 2.14a); *Spirillospora*, on the other hand, is reported to have rod-shaped spores, usually slightly bent, with a tuft of flagella in a subpolar location (Fig. 2.14b), and the speed of motility is higher than other motile actinobacterial spores (Higgins et al. 1967). The study of the detailed structure of actinobacterial spore flagella is limited, and further investigations are needed.

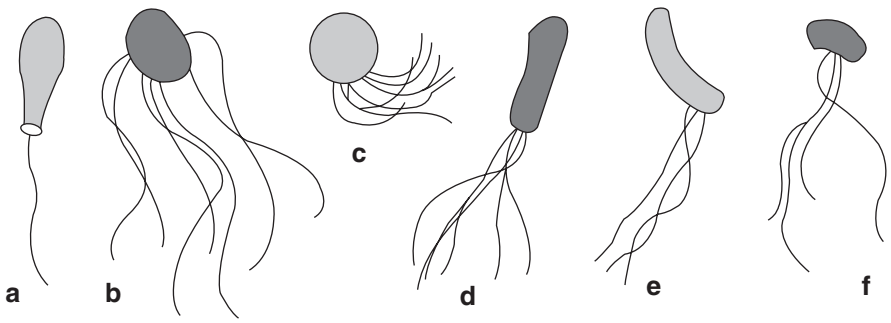

Fig. 2.13 Flagellar organizations of actinobacterial spores. (**a**) Monopolar monotrichous, (**b**) peritrichous, (**c**) polytrichous, (**d**) monopolar polytrichous (lophotrichous), (**e**) subpolar polytrichous, and (**f**) lateral polytrichous

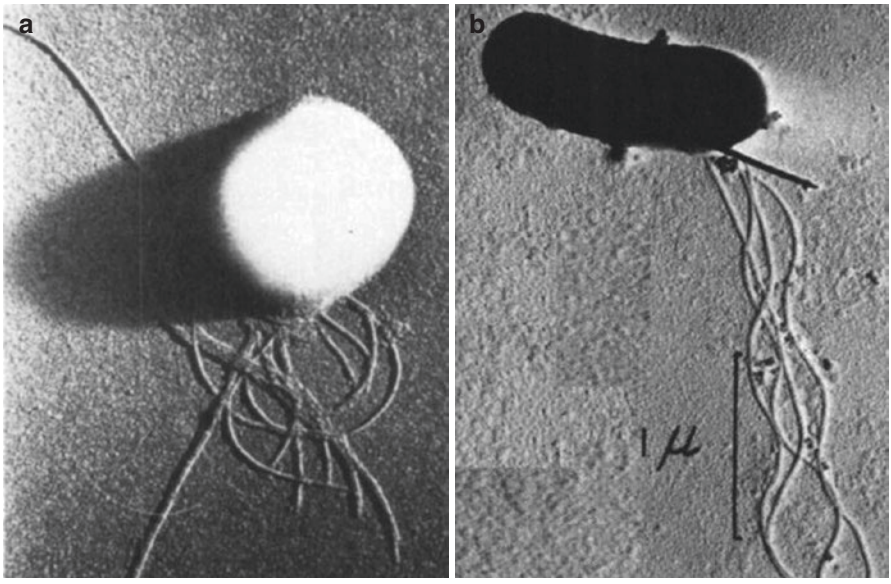

Fig. 2.14 The motile spores of (**a**) *Actinoplanes ferrugineus* (Palleroni 1979) and (**b**) *Spirillospora* sp. (Higgins et al. 1967)

Pili are protein structures that extend from the surface of bacterial cells to allow the bacteria to adhere to their environment (Gerlach and Hensel 2007).

Pili are of important virulence factors of pathogenic bacteria and oral flora since they enable these bacteria to attach the molecules on various host tissues. Moreover, only few Gram-positive bacteria have been reported to possess pili, and if they elaborate these filaments, they have shown to be both shorter and thinner than pili of Gram-negative pathogens (Ton-That and Schneewind 2003). However, there are many members within actinobacteria able to elaborate pili. Pathogen *Corynebacterium diphtheriae* contains adhesive pili on its surface which play prominent roles in its adhesion to different host tissues. There are also genes of the subunits of adhesive pili discovered in *Corynebacterium ulcerans* genome. Pili are important in interbacterial interactions between oral *Streptococci* and *Actinomyces* in the mouth. *A. viscosus* and *A. naeslundii* have two types of pili where one of them is involved in attachment of the bacteria to hard surfaces in the mouth and the other is responsible for the coaggregation reactions with other bacteria (Bowden 1996). *Actinomyces oris* possess pili which comprise two subunits, namely, FimA and FimB, the fimbrillins of shaft and tip, respectively. These subunits can be important targets for exploring novel intervention strategies to control plaque biofilm formation (Mishra et al. 2010).

Different pilus structures can be also formed in a bacterium; *C. diphtheriae* were believed to assemble a pilus structure comprising the subunits SpaA and two minor proteins, SpaB and SpaC; however, later a different pilus type containing the different subunits SpaD, SpaE, and Spa was characterized in this bacteria which are independently assembled and completely different to that of SpaABC (Gaspar and Ton-That 2006).

Generally, pilus subunit molecules (pilins) are localized by non-covalent association within the cell wall envelope specifically PG in Gram-positive bacteria and outer membrane in Gram-negatives. The length of most actinobacterial pili ranged from 0.2 to 3 μm with the diameter of 2–6 nm (Yanagawa and Honda 1976).

Conclusions

Actinobacteria, the highly evolved Gram-positive bacterial taxon, representing complex life cycle, are abundant in various aqueous and terrestrial environments and have become well adapted to live in these situations via different strategies whether on the genome-scale regulations or cellular structural properties. Although great developments are observed in the field of gene regulation studies as well as studies of actinobacterial applications and biotechnology, the consideration of methods for investigation of basic biological concepts of these bacteria such as their cell morphology and structure needs more attention. Various studies should be conducted to uncover the cell biology of especially less-studied actinobacteria to finally acquire the ability of better understanding their potentials.