

Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria

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Received: 21 May 2010 / Revised: 24 September 2010 / Accepted: 26 September 2010 / Published online: 13 October 2010
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Abstract Endophytic actinobacteria, which exist in the inner tissues of living plants, have attracted increasing attention among taxonomists, ecologists, agronomists, chemists and evolutionary biologists. Numerous studies have indicated that these prolific actinobacteria appear to have a capacity to produce an impressive array of secondary metabolites exhibiting a wide variety of biological activity, such as antibiotics, antitumor and anti-infection agents, plant growth promoters and enzymes, and may contribute to their host plants by promoting growth and enhancing their ability of withstanding the environmental stresses. These microorganisms may represent an underexplored reservoir of novel species of potential interest in the discovery of novel lead compounds and for exploitation in pharmaceutical, agriculture and industry. This review focuses on new findings in the isolation methods, bio- and chemical diversity of endophytic actinobacteria and reveals the potential biotechnological application. The facing problems and strategies for biodiversity research and bioactive natural products producing are also discussed.

Keywords Endophytic actinobacteria · Biodiversity · Natural products · Biotechnology

Introduction

Over the last several decades, natural products have continued to play a highly significant role in the drug discovery and development process. As reviewed by Newman and Cragg (2007), about 28% of the new chemical entities and 42% of the anticancer drugs introduced into the market worldwide from 1981 to 2006 were natural products and their derivatives. Besides plants, microorganisms represent a rich source of bioactive metabolites. More than 22,000 biologically active compounds have been obtained from microbes by the end of 2002. Among them, 45% were produced by actinobacteria, especially the excellent producers in the genus *Streptomyces* (Bérdy 2005). Actinobacteria have made a phenomenal contribution to the health and well-being of people throughout the world (Demain and Sanchez 2009). The emergence of antibiotics resistance developed in bacterial pathogens and the current increase in the number of new diseases and pathogens, such as acquired immunodeficiency syndrome, severe acute respiratory syndrome and H1N1 flu virus has caused a resurgence of interest in finding new biologically active compounds for drug discovery. However, for many years intensive screening of soil born microbes, the frequency of discovering structurally new compounds is apparently decreasing (Bérdy 2005). Thus, unexplored and new microbial habitats need to be examined for microbial resources that produce useful bioactive compounds.

One relatively overlooked and promising niche is the inner tissues of higher plants. Early studies have demonstrated that some actinobacteria can form intimate associations with plants

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and colonize their inner tissues. *Frankia* species and *Streptomyces scabies* can penetrate their host and establish either endophytic or pathogenic associations (Benson and Silvester 1993; Doumbou et al. 1998). The actinomycete bacteria that reside in the tissue of living plants and do not visibly harm the plants are known as endophytic actinobacteria (Stone et al. 2000). These microbes live in different organs (roots, stems, leaves, flowers, fruits and seeds) of the host plants, mainly in inter or intracellular spaces. It is noteworthy that, of the nearly 300,000 plant species on the earth, each individual plant is considered to host one or more type of endophytes (Strobel and Daisy 2003), creating an enormous biodiversity. However, only a few of these plants-associated endophytic actinobacteria have been studied, indicating the opportunity to find interesting species and related natural products among myriads of plants in different niches and ecosystems is great. Recent studies have revealed a large richness of endophytic actinobacterial species and diverse compounds with different functions (Araujo et al. 2002; Coombs and Franco 2003; Ryan et al. 2008; Bascom-Slack et al. 2009). In some cases, they can act as biological control agents (Cao et al. 2005), enhance plant growth (Igarashi et al. 2002) and promote plant establishment under adverse conditions (Hasegawa et al. 2006). These actinobacteria are relatively unstudied and potential sources of novel natural products for exploitation in medicine, agriculture, and industry (Strobel et al. 2004). The goals of this review are to summarize isolation and cultivation methods and new findings in the recent study of the astounding endophytic actinobacterial diversity and discuss the enormous biotechnological potential in the areas of natural products discovery and related applications.

Isolation and cultivation methods for endophytic actinobacteria

Early in the year 1886, the genus *Frankia* was isolated from non-legume root nodules, indicating that actinomycetes can closely be associated with plants (Okazaki 2003). For the last two decades, non-*Frankia* endophytic actinobacteria have been isolated from almost all vascular plants examined, ranging from woody species to herbaceous plants. It is evident that colonization of terrestrial plants by actinobacteria is ubiquitous and common in nature. Thus, endophytic actinobacteria are important components of microbial biodiversity because of the numerous plant species. Although, theoretically, each plant can be selected for actinobacteria isolation. Strobel and Daisy (2003) stressed that plant selection is tactical. Those plants with an unconventional setting and biology as well as with established ethnobotanic values would be preferred and promising sources of endophytes producing novel bioactive

products. A specific protocol for isolation of actinobacteria from a given plant is important, as isolation represents the most crucial step during the process of obtaining pure cultures, and the fact that host species, sampling strategy, host-endophyte and inter-endophyte interactions, tissue type and ages, geographic and habitat distribution, culture conditions, surface sterilants and selective media all influence the detection and enumeration of endophytes (Zhang et al. 2006). Some detailed isolation methods and procedures, including plant sampling, surface sterilization and used media have been reviewed (Hallmann et al. 2006) and introduced by Coombs and Franco (2003).

Surface sterilization is the first and obligatory step for endophyte isolation in order to kill all the surface microbes. It is usually accomplished by treatment of plant tissues with oxidant or general sterilant for a period, followed by a sterile rinse. Commonly used surface disinfectants are ethanol (70–95%), sodium hypochlorite (3–10%) and hydrogen peroxide. Some surfactants such as Tween 20, Tween 80 and Triton X-100 can be added to enhance surface sterilization effectiveness (Sturz 1995; Hallmann et al. 2006). A common protocol involves a three-step procedure as described by Coombs and Franco (2003). We recommend a five-step procedure, and adding sodium thiosulfate solution after being treated by sodium hypochlorite to improve cultivation efficiency on media plates, due to the fact that thiosulfate can suppress the detrimental effects of residual NaOCl on plant material surfaces, which may kill endophytes or at least stressed them so much that they become unable to form colonies on the plates (Qin et al. 2009b). After the treatment, plant tissues can be soaked in 10% NaHCO₃ solution in order to inhibit the endophytic fungi, which could overgrow the samples and mask the actinobacteria (Nimnoi et al. 2010). After each treatment, validation of surface sterilization should be checked to prove that the epiphytic microorganisms have been killed and that subsequent isolates are true endophytes. In general, the sterilization procedure should be optimized for each plant tissues, especially the sterilization time, since the sensitivity varies with plant species, age and organs.

Pretreatment of plant tissues is an important step for isolation of endophytic actinobacteria. Surface-sterilized plant samples often need drying at 80°C or 100°C for 15 to 30 min to kill bacteria. Commonly, materials were aseptically sectioned into small fragments about 0.2×1.0 cm (Coombs and Franco 2003; Cao et al. 2004; Verma et al. 2009a, b; Fialho de Oliveira et al. 2010), then distributed onto isolation media. On this basis, tissues can be aseptically crumbled into smaller fragments by commercial blender (Qin et al. 2008a, b; Li et al. 2009d), which enlarged the colonization area of samples onto the media agar, thus making it helpful for recovering endophytes. Tender and soft tissues can be pestled and homogenized in a mortar with extraction solution or buffer and then use gradient dilution method. Maceration,

vacuum and pressure bomb techniques have been employed for isolating gram-positive species (Hallmann et al. 1997a, b). These two preferred methods could recover a higher number of less commonly detected genera. We also recommend the newly applied different combined enzymatic hydrolysis and differential centrifugation bacterial cell enrichment methods (Jiao et al. 2006; Qin et al. 2009b; Ikeda et al. 2009). These techniques collected most of the microbes associated with plant tissues and maintained the microbial cells intact, and were especially useful for isolating rare endophytic actinobacteria. In a word, to make endophytes complete release from inner parts of plant material is a significant step for pure culture isolation. Based on this rationale, some other innovative pre-treatment methods can be explored.

Growth of microbes in the laboratory is dependent on the composition of the media and the cultivation conditions that are applied. For endophytic actinobacteria, some classical media for soil actinomycetes isolation, such as humic acid-vitamin (HV) (Hayakawa 1990), International *Streptomyces* Project media ISP 2 and ISP 5 (Shirling and Gottlieb 1966), raffinose-histidine agar (Vickers et al. 1984) and starch casein agar (Küster and Williams 1964) are also available. Low nutrient medium TWYE was found effective for isolation of endophytic actinobacteria (Coombs and Franco 2003; Qin et al. 2009b; Li et al. 2009a), due to the fact that high nutrient concentration allowed fast growing bacteria to overgrow slower growing actinobacteria. Recently, by our large-scale isolation investigations of tropical rainforest plants, some media composed of amino acids (proline, arginine, and asparagine) as nitrogen sources and cellulose, xylan, sodium propionate, sodium succinate as carbon sources had prominent isolation effectiveness, especially for some uncommon rare endophytic actinobacterial genera (Qin et al. 2009b). This could be explained by the fact that in many plants amino acids account for the major nitrogen, and cellulose, xylan are the components of plant cell wall. Sometimes, different pre-treatment methods and media combinations had different isolation effectiveness. We also found that adding a certain amount of plant extracts into the isolation medium is effective. This may be due to the different physiological properties of some actinomycetes in plant tissues and soils (Okazaki 2003). At present, understanding the physiological properties of endophytic actinobacteria seems difficult because of the complicated symbiotic relationships. By simulating the micro-environments of inner plants to design media is a good strategy for isolation.

Biodiversity of endophytic actinobacteria

Endophytic actinobacteria have been isolated from a variety of healthy plant species ranging from crop plants, such as

wheat, rice, potato, carrots, tomato and citrus (Nejad and Johnson 2000; Araujo et al. 2002; Coombs and Franco 2003; Surette et al. 2003; Sessitsch et al. 2004; Tian et al. 2007; Velazquez et al. 2008), different woody tree species (Taechowisan et al. 2003; Zin et al. 2007; Yuan et al. 2008; Zhao et al. 2010a, b, c), ferns and club mosses (Janso and Carter 2010). In general, *Streptomyces* spp. were the most predominant species and *Microbispora*, *Micromonospora*, *Nocardioides*, *Nocardia* and *Streptosporangium* are the common genera. For instance, 619 actinomycetes were isolated from different cultivars of tomato, and all of them were *Streptomyces* spp. (Tan et al. 2006). From 36 medicinal plant species of Thailand, Taechowisan et al. (2003) isolated 330 strains belonging to four different genera (*Streptomyces*, *Microbispora*, *Nocardia* and *Micromonospora*). Lee et al. (2008) isolated 81 endophytic actinobacteria including eight genera from Chinese cabbage roots, and *Microbispora* spp. were the most common isolates, followed by *Streptomyces* spp. and *Micromonospora* spp. Relative to stems and leaves, roots harboured more diverse actinobacterial populations. To date, more than 40 new taxa have been found by polyphasic taxonomic approaches, including four new genera, *Plantactinospora*, *Actinophytocola*, *Phytohabitans* and *Jishengella* (Table 1). Most of these published species had more than 97% similarities with recognized nearest relatives based on the analysis of the 16S rRNA gene sequences with closest non-endophytic neighbours. In addition, few species possessed unique rRNA gene sequences, which probably had experienced long-time evolution. This may presume that some actinobacterial endophytes are very similar with non-endophytic isolates, especially those from soils. In fact, the endophyte population was considered to be a subset of the rhizosphere population and soil type, soil microbial population significantly affects the endophytic actinobacterial communities (Conn and Franco 2004; Compant et al. 2010).

Strobel and Daisy (2003) believed that the greatest diversity of endophytes is likely to occur in the tropical and temperature regions. Janso and Carter (2010) isolated 123 endophytic actinomycetes from tropical plants collected from several locations in Papua New Guinea and Mborokua Island, Solomon Islands. 16S rDNA sequence analysis revealed that 17 different genera were represented and rare genera such as *Sphaerisporangium* and *Planotetraspora*, which have never been previously reported to be endophytic, were quite prevalent. Phylogenetic analyses suggested that the many endophytic strains in *Thermomonosporaceae* and *Micromonosporaceae* may represent new genera. Tropical rainforests possessing the greatest biodiversity on the earth have the prospect of housing endophytes with great biodiversity. In our study, 2,174 endophytic actinobacteria were isolated from different

Table 1 Novel actinobacteria isolated from endophytic environments

GenBank accession no.	Name of the endophytes	Host plants	Closest non-endophytic species and 16S rRNA gene sequences similarity (%)	Reference
AJ784008	<i>Micromonospora coriariae</i>	<i>Coriaria myrtifolia</i>	<i>M. endolithica</i> (98.94%)	Trujillo et al. 2006
AJ783996	<i>Micromonospora lupini</i>	<i>Lupinus angustifolius</i>	<i>M. mirobrigensis</i> (98.5%)	Trujillo et al. 2007
AJ783993	<i>Micromonospora saelicesensis</i>	<i>Lupinus angustifolius</i>	<i>M. purpureochromogenes</i> (98.7%)	Trujillo et al. 2007
AM944497	<i>Micromonospora pisi</i>	<i>Pisum sativum</i>	<i>M. pattaloongensis</i> (98.7%)	Garcia et al. 2010
DQ343154	<i>Pseudonocardia oroxyli</i>	<i>Oroxylum indicum</i>	<i>P. halophobica</i> (97.8%)	Gu et al. 2006
EU921261	<i>Pseudonocardia acaciae</i>	<i>Acacia auriculiformis</i> A. Cunn. ex Benth.	<i>P. spinospora</i> (96.2%)	Duangmal et al. 2009
DQ887489	<i>Pseudonocardia endophytica</i>	<i>Lobelia clavata</i>	<i>P. kongjuensis</i> (98.5%)	Chen et al. 2009b
GQ906587	<i>Pseudonocardia tropica</i>	<i>Maytenus austroyunnanensis</i>	<i>P. alni</i> (99.5%)	Qin et al. 2009g
FJ805427	<i>Pseudonocardia adelaidensis</i>	<i>Eucalyptus microcarpa</i>	<i>P. zijingensis</i> (98.7%)	Kaewkla and Franco 2010a
FJ805426	<i>Pseudonocardia eucalypti</i>	<i>Eucalyptus microcarpa</i>	<i>P. spinospora</i> (96.3%)	Kaewkla and Franco 2010b
GU227146	<i>Pseudonocardia artemisiae</i>	<i>Artemisia annua</i> L.	<i>P. saturnea</i> (96.6%)	Zhao et al. 2010a
HM153789	<i>Pseudonocardia sichuanensis</i>	<i>Jatropha curcas</i> L.	<i>P. zijingensis</i> (98.6%)	Qin et al. 2010b
EU375845	<i>Dietzia schimae</i>	<i>Schima</i> sp.	<i>D. maris</i> (99.8%)	Li et al. 2008b
EU375846	<i>Dietzia cercidiphylli</i>	<i>Cercidiphyllum japonicum</i>	<i>D. natronolimnaea</i> (99.5%)	Li et al. 2008b
EU200681	<i>Glycomyces endophyticus</i>	<i>Carex baccans</i> Nees	<i>G. algeriensis</i> (99%)	Qin et al. 2008b
DQ460469	<i>Glycomyces sambucus</i>	<i>Sambucus adnata</i> Wall	<i>G. lechevalierae</i> (97.2%)	Gu et al. 2007
EU200682	<i>Glycomyces scopariae</i>	<i>Scoparia dulcis</i>	<i>G. algeriensis</i> (97.4%)	Qin et al. 2009a
EU814511	<i>Glycomyces mayteni</i>	<i>Maytenus austroyunnanensis</i>	<i>G. algeriensis</i> (97.1%)	Qin et al. 2009a
DQ460470	<i>Streptomyces alni</i>	<i>Alnus nepalensis</i> D. Don	<i>S. hebeiensis</i> (97.6%)	Liu et al. 2009
EU200685	<i>Streptomyces artemisiae</i>	<i>Artemisia annua</i> L.	<i>S. armeniacus</i> (99.9%)	Zhao et al. 2010b
EU925562	<i>Streptomyces sedi</i>	<i>Sedum</i> sp.	<i>S. specialis</i> (97.5%)	Li et al. 2009d
EU200683	<i>Streptomyces mayteni</i>	<i>Maytenus austroyunnanensis</i>	<i>S. phaeopurpureus</i> (99.5%)	Chen et al. 2009a
GU367158	<i>Nonomuraea endophytica</i>	<i>Artemisia annua</i> L.	<i>N. candida</i> (98.8%)	Li et al. 2010b
FJ157184	<i>Nonomuraea antimicrobica</i>	<i>Maytenus austroyunnanensis</i>	<i>N. candida</i> (98.2%)	Qin et al. 2009d
EU814512	<i>Saccharopolyspora endophytica</i>	<i>Maytenus austroyunnanensis</i>	<i>S. flava</i> (97.7%)	Qin et al. 2008a
EU005371	<i>Saccharopolyspora gloriosae</i>	<i>Gloriosa superba</i> L.	<i>S. gregorii</i> (99.1%)	Qin et al. 2010a
FJ214364	<i>Saccharopolyspora tripterygii</i>	<i>Tripterygium hypoglaucom</i>	<i>S. flava</i> (97.6%)	Li et al. 2009b
EU429322	<i>Actinoallomurus acaciae</i>	<i>Acacia auriculiformis</i> A. Cunn. ex Benth	<i>A. caesius</i> (99.3%)	Thamchaipenet et al. 2010
EU420071	<i>Actinoallomurus oryzae</i>	<i>Oryza sativa</i> L. cv. KDML 105	<i>A. iriomotensis</i> (99.2%)	Indananda et al. 2010a, b
EU005372	<i>Micrococcus endophyticus</i>	<i>Aquilaria sinensis</i>	<i>M. luteus</i> (99.06%)	Chen et al. 2009c

Table 1 (continued)

GenBank accession no.	Name of the endophytes	Host plants	Closest non-endophytic species and 16S rRNA gene sequences similarity (%)	Reference
FJ214355	<i>Micrococcus yunnanensis</i>	<i>Polyspora axillaris</i>	<i>M. luteus</i> (99.7%)	Zhao et al. 2009
FJ805428	<i>Nocardia callitridis</i>	<i>Callitris preissii</i>	<i>N. nova</i> (97.4%)	Kaewkla and Franco 2010a, b, c
HM153801	<i>Nocardia endophytica</i>	<i>Jatropha curcas</i> L.	<i>N. nova</i> (97.5%)	Xing et al. 2010
EF466117	<i>Nocardioides caricicola</i>	<i>Carex scabrifolia</i> Steud	<i>N. pyridinolyticus</i> (97.0%)	Song et al. 2010
FJ157185	<i>Actinomadura flavalba</i>	<i>Maytenus austroyunnanensis</i>	<i>A. atramentaria</i> (97.4%)	Qin et al. 2009e
EU420070	<i>Actinophytocola oryzae</i>	<i>Oryza sativa</i> L. cv. RD6	<i>Kibdelosporangium aridum</i> (95.5%)	Indananda et al. 2010a, b
FJ214343	<i>Plantactinospora mayteni</i>	<i>Maytenus austroyunnanensis</i>	<i>M. endolithica</i> (98.1%)	Qin et al. 2009c
AB490769	<i>Phytohabitans suffuscus</i>	Orchid	<i>M. pattaloongensis</i> (97.7%)	Inahashi et al. 2009
FJ157186	<i>Jiangella alba</i>	<i>Maytenus austroyunnanensis</i>	<i>J. alkaliphila</i> (98.8%)	Qin et al. 2009f
EU560726	<i>Jishengella endophytica</i>	<i>Acanthus illicifolius</i>	<i>M. olivasterospora</i> (98.7%)	Xie et al. 2010
FJ214356	<i>Herbidospora osyris</i>	<i>Osyris wightiana</i> Wall. ex Wight	<i>H. cretacea</i> (99.9%)	Li et al. 2009c
FJ214362	<i>Kineosporia mesophila</i>	<i>Tripterygium wilfordii</i>	<i>K. mikuniensis</i> (98.2%)	Li et al. 2009a
DQ473536	<i>Leifsonia ginsengi</i>	Ginseng	<i>L. poae</i> (97.6%)	Qiu et al. 2007
EU325542	<i>Rhodococcus cercidiphylli</i>	<i>Cercidiphyllum japonicum</i>	<i>R. fascians</i> (99.6%)	Li et al. 2008a

medicinal plants in Xishuangbanna tropical rainforest of China by using diverse pretreatment methods and selective media, and they represent an unexpected variety of ten different suborders and 32 genera, including at least 19 new taxa (Qin et al. 2009b). Only from one kind of plant, *Maytenus austroyunnanensis*, a new genus and seven new species were isolated. Obviously, the actinobacterial richness and diversity from tropical rainforest are much higher comparison with other regions, and tropical rainforest have potential as promising source of actinobacteria and new species. The endophytic actinobacterial communities are diverse and the extent of diversity may vary between different sample collection regions and different plant species. The diversity of genera and the number of culturable endophytic actinobacteria was largely dependent on the isolation methods.

It has been estimated that less than 1% of bacteria species are currently known (Davis et al. 2005), indicating that millions of microbial species remain to be discovered. The applications of 16S rRNA gene-based culture-independent molecular approaches, such as polymerase chain reaction (PCR)-based 16S rRNA gene clone library,

denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism (T-RFLP) analysis are useful to reveal the complex microbial community inhabiting various plants. Sometimes, the combination of culturing methods and culture-independent analysis is needed for the study of endophytic community. Conn and Franco (2004) investigated the wheat root actinobacteria using the T-RFLP method and revealed a large diversity than that obtained by culturing. The presence of endophytic actinobacteria from rice was studied by Tian et al. (2007), and their results suggest diverse communities within stems and roots. Jiao et al. (2006) firstly introduced the idea of enriching uncultured bacterial cells from plant tissues by enzymatic hydrolysis of the plant cell wall, followed by differential centrifugation. Subsequently, Wang et al. (2008) reported the diversity of uncultured microbes associated with medicinal plant *Mal-lotus nudiflorus* using a modified method, and found that actinobacteria were the most dominant microbial group, covering 37.7% in the 16S rRNA gene library. This enzymatic hydrolysis and differential centrifugation microbial enrichment method has also been carried out to study the endophytic bacterial diversity in grapevine leaf tissues

(Bulgari et al. 2009). Another technique suitable for enriching bacterial cells from fresh plant tissues was developed (Ikeda et al. 2009) and has been successfully applied to analyze the clone libraries of 16S rRNA gene and clarify the diversity of endophytic actinobacterial communities in stems and leaves of soybeans and rice (Ikeda et al. 2009, 2010). We studied the composition of endophytic actinobacteria associated with *M. austroyunnanensis* using both culture-based and culture-independent techniques. In total, 20 different genera were isolated from roots, stems and leaves, with *Streptomyces* spp. the most predominant species, whereas more than 30 genera and uncultured actinobacteria were detected from the 16S rRNA gene clone library using the cell enrichment method, and the culturable community composition was different from that of the 16S rRNA gene clone library (unpublished data). However, detection limits of these techniques still exist and may prevent the identification of many heterogeneous species. Recently developed high-throughput sequencing technologies, including those commercialized by 454 Life Sciences/Roche Applied Sciences (454), Illumina Incorporated (Solexa), Applied Biosciences (SOLiD), Dover Systems (Polonator), and Helicos Biosciences Corporation have allowed for rapid sequencing of whole genomes and been used to explore a variety of microbial ecological communities (Mardis 2008; Shendure and Ji 2008; Miller et al. 2009; Lauber et al. 2010; Robinson et al. 2010). In 2010, pyrosequencing has also been used for the first time to examine the bacterial endophyte community in the roots of 12 different potato cultivars revealing an unprecedented level of bacterial root endophytes (Manter et al. 2010). These discoveries greatly improved our understanding of the ecological distributions and the complexity of plant-associated actinobacteria. However, the actual number and diversity of endophytic actinobacteria are enormous and a majority remains unknown.

Bioactive natural products and biotechnological potential

From a drug discovery point of view, the novel actinobacterial strains are attractive, as they are likely to contain new genes in theory and held promising for novel products, thus the chance of finding novel pharmaceutical bioactive compounds from endophytic actinobacteria is considerable. Endophytic actinobacteria associated with traditionally used medicinal plants especially of the tropics could be a rich source of functional metabolites (Strobel et al. 2004). Current interest in natural products from endophytes especially endophytic fungi is evident from the number of review articles that have appeared in the recent literatures (Hasegawa et al. 2006; Zhang et al. 2006; Gunatilaka 2006;

Guo et al. 2008; Staniek et al. 2008; Ryan et al. 2008; Verma et al. 2009a, b). Recent studies of the culturable endophytic actinobacteria have resulted in the identification of many new natural products with diverse biological activities (Table 2). This review focuses particularly on the recent advances up to the writing time of 2010. In this section, we mainly review the bioactive compounds from endophytic actinobacteria and their biotechnological potential applications. Some examples are listed below.

Antibiotics from endophytic actinobacteria

Many endophytic actinobacteria, especially those from medicinal plants possess the ability of inhibiting or killing a wide variety of harmful microorganisms like pathogenic bacteria, fungi and viruses. Thus, there is great application value to develop antimicrobial drugs from endophytic actinobacteria. Hitherto, a lot of new antibiotics have been isolated, such as munumbicins A-D (Castillo et al. 2002), celastramycins A-B (Pullen et al. 2002), kakadumycins (Castillo et al. 2003) and demethylnovobiocins (Igarashi 2004). From the culture broth of endophytic strain *Streptomyces* sp. TP-A0595, a simple compound 6-prenylindole was isolated and exhibited significant antifungal activity against plant pathogen *Fusarium oxysporum*. 6-Prenylindole was originally isolated from the plant liverwort (*Hepaticae*), and this is an additional example of the isolation of the same compound from plant and endophyte (Igarashi 2004). Two novel compounds cedarmycins A and B were isolated from the strain *Streptomyces* sp. TP-A0456, which was isolated from a twig of cedar. Cedarmycins A showed in vitro antifungal activity against *Candida glabrata* with the MIC value of 0.4 µg/ml (Igarashi 2004). An endophytic *Streptomyces* sp. Tc022 isolated from roots of *Alpinia galanga* strongly inhibited *Colletotrichum musae* and *Candida albicans*. Extraction of the culture medium of *Streptomyces* sp. Tc022 afforded a major component actinomycin D, which displayed very strong antifungal activity (Taechowisan et al. 2006). Castillo et al. (2006) isolated two new chromophoric peptides antibiotics, designated munumbicins E-4 and E-5 from endophytic *Streptomyces* NRRL 30562, which also produced broad-spectrum antibiotics munumbicins A-D. Both compounds showed broad-spectrum activity against gram-positive and gram-negative bacteria. Recently, a new antimycotic compound saadamycin was isolated from endophytic *Streptomyces* sp. Hedaya48, and it exhibited significant antimycotic activity against dermatophytes and other clinical fungi (El-Gendy and EL-Bondkly 2010). These studies listed above, reinforced the assumption that endophytic actinobacteria could be a promising source of antimicrobial substances.

Table 2 Novel bioactive compounds isolated from endophytic actinobacteria

Endophytes	Host	Bioactive compounds	Type	Activity	Reference
<i>Streptomyces</i> sp. NRRL 30562	<i>Kennedia nigriscans</i>	Munumbicins A-D	Peptides	Antibiotic	Castillo et al. 2002
<i>Streptomyces</i> sp. NRRL 30566	<i>Grevillea pteridifolia</i>	Kakadumycins	Peptides	Antibiotic	Castillo et al. 2003
<i>Streptomyces</i> sp. MSU-2110	<i>Monstera</i> sp.	Coronamycins	Peptides	Antibiotic	Ezra et al. 2004
<i>Streptomyces</i> sp. NRRL 30562	<i>K. nigriscans</i>	Munumbicins E-4 and E-5	Peptides	Antibiotic	Castillo et al. 2006
<i>Streptomyces</i> sp. CS	<i>Maytenus hookeri</i>	24-demethyl-bafilomycin C ₁	Macrolides	Antimicrobial, antitumor	Lu and Shen 2003
<i>Streptomyces</i> sp. CS	<i>M. hookeri</i>	24-demethyl-bafilomycin A2	Macrolides	Antimicrobial, antitumor	Lu and Shen 2004
<i>Streptomyces</i> sp. CS	<i>M. hookeri</i>	24-demethyl-bafilomycin A1; 21-O-methyl-24-demethyl-bafilomycin A1; 19,21-di-O-methyl-24-demethyl-bafilomycin A1; 17,18-dehydro-19,21-di-O-methyl-24-demethyl-bafilomycin A1; 24-demethyl-bafilomycin D	Macrolides	Antitumor	Li et al. 2010a
<i>Streptomyces albidoflavus</i>	<i>Bruguiera gymnorhiza</i>	Antimycin A ₁₈	Macrolides	Antifungal	Yan et al. 2010
<i>Streptomyces</i> sp. ls9131	<i>M. hookeri</i>	Dimeric dinactin and Dimeric nonactin	Macrotetrolide	Antimicrobial, antitumor	Zhao et al. 2005
<i>Streptomyces</i> sp. TP-A0595	<i>Allium tuberosum</i>	6-Prenylindole	Alkaloids	Antifungal	Igarashi 2004
<i>Streptomyces</i> sp. TP-A0556	<i>Aucuba japonica</i> Thunb	Demethylnovobiocins	Coumarins	Antimicrobial	Igarashi 2004
<i>Streptomyces griseus</i> subsp.	<i>Kandelia candel</i>	7-(4-aminophenyl)-2,4-dimethyl-7-oxohept-5-enoic acid; 9-(4-aminophenyl)-7-hydroxy-2,4,6-trimethyl-9-oxo-non-2-enoic acid; 12-(4-aminophenyl)-10-hydroxy-6-(1-hydroxyethyl)-7,9-dimethyl-12-oxo-dodeca-2,4-dienoic acid	<i>p</i> -Aminoacetophenonic acids	Antimicrobial	Guan et al. 2005
<i>Streptomyces laceyi</i>	<i>Ricinus communis</i> L.	Salaceyins A and B	6-Alkylsalicylic acids	Antitumor	Kim et al. 2006
<i>Streptomyces hygrosopicus</i> TP-A0451	<i>Pteridium aquilinum</i>	Pterocidin	δ-lactone	Antitumor	Igarashi et al. 2006
<i>Streptomyces</i> sp. CS	<i>Maytenus hookeri</i>	Naphthomycin K	Ansamycins	Antitumor	Lu and Shen 2007
<i>Streptomyces aureofaciens</i> CMUAc 130	<i>Zingiber officinale</i> Rosc.	5,7-dimethoxy-4- <i>p</i> -methoxyphenylcoumarin; 5,7-dimethoxy-4-phenylcoumarin	Arylcoumarins	Antifungal, antitumor, anti-inflammatory	Taechowisan et al. 2005, 2007a, b
<i>Micromonospora lupini</i>	<i>Lupinus angustifolius</i>	Lupinacidins A and B	Anthraquinones	Antitumor	Igarashi et al. 2007
<i>Streptomyces</i> sp. SUC1	<i>Ficus benjamina</i>	Lansai B and C	Phenols	Weakly anticancer anti-inflammatory	Tuntiwachwuttikul et al. 2008; Taechowisana et al. 2009
<i>Streptomyces</i> sp. TP-A0456	<i>Cryptomeria japonica</i>	Cedarmycins A and B	Butyrolactones	Antifungal	Igarashi 2004
<i>Streptomyces</i> sp. MaB-QuH-8	<i>Maytenus aquifolia</i> Mart.	Celastramycins A and B	Heterocyclic compounds	Antimicrobial	Pullen et al. 2002
<i>Streptomyces</i> sp. Hedaya48	<i>Aplysina fistularis</i> (sponge)	Saadamycin	Heterocyclic compound	Antifungal	El-Gendy and EL-Bondkly 2010

Antitumor and anti-inflammatory agents from endophytic actinobacteria

In recent years, there has been an increasing interest in searching antitumor agents from endophytes. Considering that endophytic actinobacteria live in close association with their host plants and the long co-evolution relationship, there is a real possibility that genes involved in natural products biosynthesis could be exchanged via horizontal gene transfer (HGT) between microbes and plants, resulting in production of plant-derived compounds by a microbe such as the paclitaxel-producing *Kitasatospora* sp. isolated from *Taxus baccata* in Italy (Caruso et al. 2000; Janso and Carter 2010). This is the first report of production of taxol from endophytic actinomycetes. Another example is the maytansinoids (19-membered macrocyclic lactams related to ansamycin antibiotics), extraordinarily potent antitumor agents that were originally isolated from members of the higher plant families *Celastraceae*, *Rhamnaceae* and *Euphorbiaceae* (Kupchan et al. 1972; Powel et al. 1982), as well as some mosses (Suwanborirux et al. 1990), and, remarkably, from plant-associated actinomycete *Actinosynnema pretiosum* (Higashide et al. 1977). Noticeably, one novel chlorine-containing ansamycin, namely naphthomycin K (Fig. 1a), was isolated from the endophytic strain *Streptomyces* sp. CS of the maytansinoids producer medicinal plant *Maytenus hookeri*. It showed evident cytotoxic activity against P388 and A-549 cell lines at IC_{50} 0.07 and 3.17 μ M, but no inhibitory activities against *Staphylococcus aureus* and *Mycobacterium tuberculosis* (Lu and Shen 2007). Interestingly, ansacarbamitocins, a new family of maytansinoids, were reported from a soil actinomycete strain *Amycolatopsis* CP2808 which belongs to the family *Pseudonocardiaceae*, the same family as the ansamycin-producing strain *A. pretiosum* (Snipes et al. 2007). Strain *Streptomyces* sp. CS was an outstanding bioactive compounds producer. Previously, 24-demethylbafilomycin C1, a new member of the bafilomycin subfamily and two more new bafilomycin derivatives were isolated from the this strain (Lu and Shen 2003, 2004). During the subsequent research, five new 16-membered macrolides, belonging to the bafilomycin subfamily were isolated and showed cytotoxic activity against MDA-MB-435 cell line in vitro (Li et al. 2010a). From the strain *Streptomyces* sp. ls9131, which was also isolated from *M. hookeri*, two novel macrolides were found. Bioassay results showed that the compound dimeric dinactin (Fig. 1b) had strong antineoplastic activity and antibacterial activity (Zhao et al. 2005). Two novel anthraquinones, lupinacidins A and B (Fig. 1c), were isolated from the fermentation broth of a new endophytic actinomycete *Micromonospora lupine*. Lupinacidins were found to show significant inhibitory effects on the invasion of murine colon 26-L5

carcinoma cells (Igarashi et al. 2007). Kim et al. (2006) isolated two new 6-alkylsalicylic acids, salaceyins A and B from another strain *Streptomyces laceyi* MS53 and their structures were determined on the basis of spectroscopic data (Fig. 1d). Salaceyins A and B exhibited cytotoxicity against a human breast cancer cell line (SKBR3) with IC_{50} values of 3.0 and 5.5 μ g/ml, respectively. A new cytotoxic compound, pterocidin (Fig. 1e), isolated from the endophytic *Streptomyces hygroscopicus* TP-A0451, showed cytotoxicity against some human cancer cell lines with IC_{50} values of 2.9–7.1 μ M (Igarashi et al. 2006).

Recently, two compounds 5, 7-dimethoxy-4-phenylcoumarin (Fig. 1f) and 5, 7-dimethoxy-4-*p*-methoxyphenylcoumarin, originally produced by numerous species of plants were isolated from endophytic *Streptomyces aureofaciens* CMUAc130 and showed to have antifungal and antitumor activity (Taechowisan et al. 2005, 2007a). They were also investigated for their effects not only on the formation of nitric oxide (NO), prostaglandin E2 (PGE2) and tumour necrosis factor (TNF- α), but also on inducible nitric oxide synthase and cyclooxygenase-2 in lipopolysaccharide (LPS)-induced murine macrophage RAW 264.7 cells. The inhibitory effects were shown in concentration-dependent manners. They significantly reduced the formation of TNF- α . These findings support the application of them as anti-inflammatory agent (Taechowisan et al. 2007b). From the endophytic strain *Streptomyces* sp. SUC1, four novel secondary metabolites, lansai A–D were isolated. Lansai B showed weakly active against the BC cell line (IC_{50} 15.03 μ g/ml) (Tuntiwachwuttikul et al. 2008), lansai C also showed significant anti-inflammatory activity in LPS-induced RAW 264.7 cells (Taechowisana et al. 2009). In a word, endophytic actinobacteria still remain a relatively untapped source of novel natural products, presumed to push forward the frontiers of drug discovery.

Other pharmaceutical compounds from endophytic actinobacteria

Endophytic actinobacteria also produced other bioactive compounds with diverse functions different from those discussed above. An endophytic *Streptomyces* sp. (AC-2), isolated from a traditional Chinese medicine plant *Cistanche deserticola* Y. C. Ma, produced tyrosol. This compound can promote an increase of intracellular cAMP special on GPR12 transfected cells, such as CHO and HEK293, might be a new possible ligand for GPR12 (Lin et al. 2008). A novel peptide compound coronamycin was purified from a verticillate *Streptomyces* sp. (MSU-2110) colonizing an epiphytic vine, *Monstera* sp. It exhibited remarkable active against the malarial parasite, *Plasmodium falciparum*, with an IC_{50} of 9.0 ng/ml. In addition, it

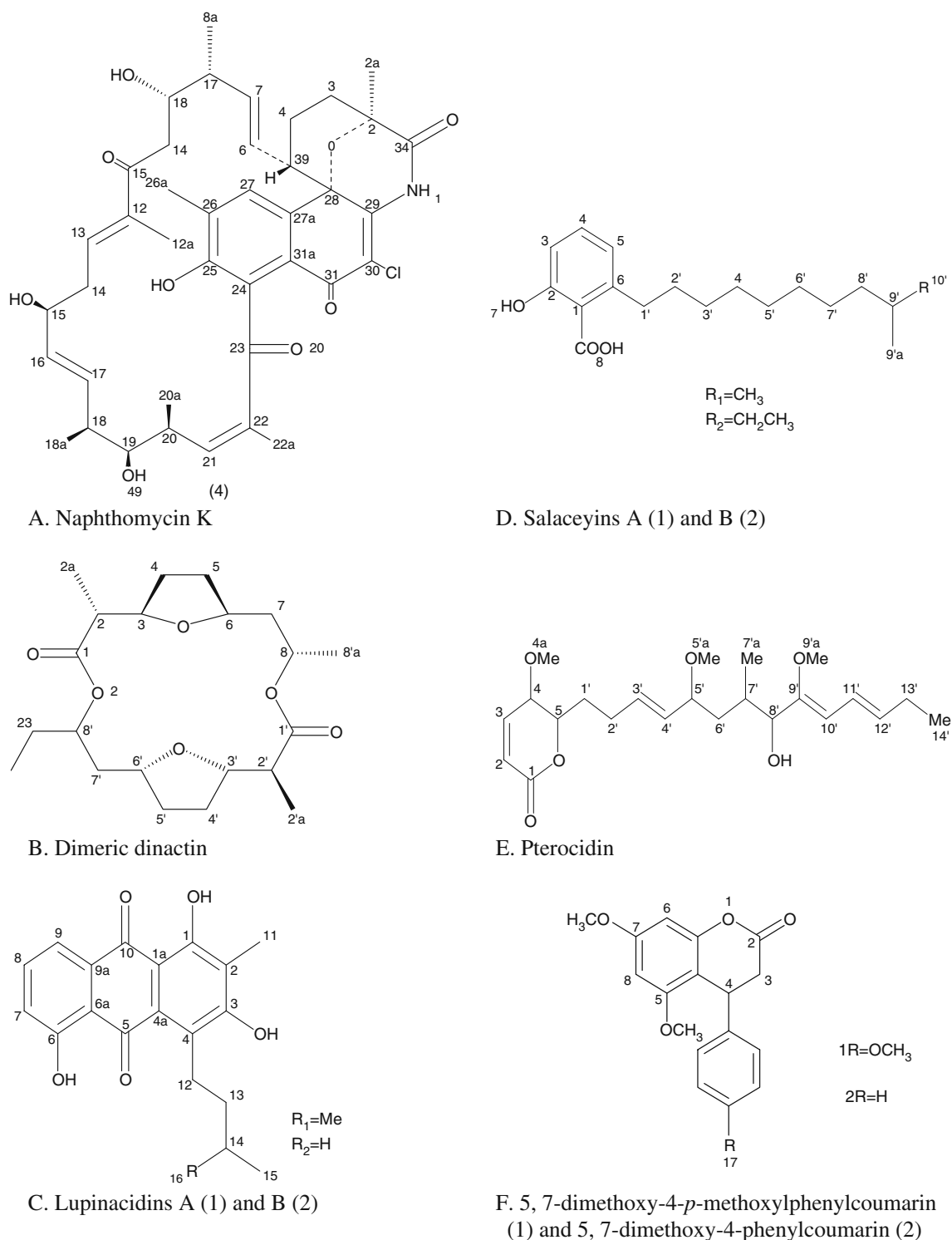


Fig. 1 Chemical structures of some representative novel bioactive compounds produced by endophytic actinobacteria

showed strong activity against pythiaceus fungi and the human fungal pathogen *Cryptococcus neoformans* (Ezra et al. 2004). These natural bioactive metabolites with multi-fold applications expand actinobacterial use arenas. Analysing the endophytic actinobacterial born compounds and their hosts (Table 2), a correlation seems to exist between

their ecological basis and bioactive metabolites. Endophytic actinomycetes in higher plants, particularly those associated with medicinal plants and being used traditional medicines, are more likely to contain bioactive compounds. This principle is supported by the examples of taxol and maytansinoids as discussed above in this review. Therefore,

to search for novel compounds could be directed towards plants that commonly serve indigenous populations for medicinal purposes and plants growing in unique environmental setting or interesting endemic locations as they are expected to harbour novel endophytes that may produce unique metabolites with diversified applications (Strobel and Daisy 2003).

Biological control agents

In the recent years, endophytic actinobacteria have attracted the attention of researchers as biological control agents of plant pathogens due to their plant colonizing ability and antifungal activities. They have been shown to protect plants against different soil-borne plant pathogens, including *Rhizoctonia solani*, *Verticillium dahliae*, *Plectosporium tabacinum*, *Gaeumannomyces graminis* var. *tritici*, *F. oxysporum*, *Pythium aphanidermatum* and *Colletotrichum orbiculare* (Krechel et al. 2002; El-Tarabily 2003; Coombs et al. 2004; Cao et al. 2005; El-Tarabily et al. 2009; Shimizu et al. 2009). Endophytic actinobacteria and their role as biocontrol agents have been partly discussed (Hasegawa et al. 2006).

Studies on the mechanisms of action of these endophytes have focused mainly on the production of bioactive compounds, such as antibiotics, cell wall degrading enzyme and competition for nutrients (El-Tarabily and Sivasithamparam 2006). In addition, endophytic actinobacteria have also been found to hold the ability of triggering plant induced systemic resistance (ISR). The ‘versatile’ endophytic strain *Streptomyces galbus* R-5 not only released cellulose, pectinase, produced actinomycin X_2 and fungichromin to induce resistance in the rhododendron seedlings, but also triggered plant jasmonate-associated defence responses (Shimizu et al. 2005). Conn et al. (2008) observed that seed inoculations with endophytic *Streptomyces* sp. EN27 and *Micromonospora* sp. strain EN43 led to increased resistance in *Arabidopsis thaliana* leaves against pathogens, *Erwinia carotovora* and *F. oxysporum*, and triggered the expression of defence genes related to SA- or jasmonic acid/ethylene-dependent signalling pathways in the absence of a pathogen. Moreover, culture filtrates obtained from cells of *Micromonospora* sp. strain EN43 grown in either minimal or rich medium activated the system acquired resistance and jasmonates/ethylene pathways, respectively, indicating that two different sets of metabolites are synthesized by this endophytic actinobacteria for eliciting the plant defense pathway (Hirsch and Valdés 2010). At present, whether or not these metabolites are antibiotics or other molecules is not known. The intimate association of endophytic actinobacteria with plants and born bioactive natural products

offers a unique opportunity to find effective drugs or biofungicides for potential application in plant protection and biological control.

Plant growth promoting agents

Nowadays, increased public concern about environmental problems caused either directly or indirectly by the use of fertilizers, pesticides, herbicides, and fungicides, has prompted researchers to consider alternatives to these established chemical strategies for facilitating plant growth in agriculture, horticulture, and silviculture (Glick et al. 2007). Endophytic actinobacteria are of special interest since they possess many properties that could benefit to plant growth. The plant growth promoting actinobacterial endophytes possess similar mechanisms for one of the following functions: production biological control agents, or production of plant growth promotion compounds, such as auxins, cytokinins and gibberellins, or producing siderophore to bind Fe^{3+} from the environment and help to improve nutrient uptake, supply of plant nutrients (nitrogen, phosphate and other mineral nutrients), or suppression of stress ethylene production by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Compant et al. 2005; Kannan and Sureendar 2008; Sun et al. 2009; Nimnoi et al. 2010). For example, several endophytic actinobacteria isolated from winter rye produced indolyl-3-acetic acid. Treatment of winter rye seeds with auxin producing strains increased the germination capacity and enhanced an intensive seedling growth in vitro (Merzaeva and Shirokikh 2010). Two compounds pteridic acids A and B were found from species *S. hygrosopicus* TP-A0451 isolated from a stem of bracken, *Pteridium aquilinum* (Igarashi 2004). Pteridic acids inhibited the rice germination at 100 ppm, but pteridic acid A promoted the root elongation at 20 ppm. Furthermore, pteridic acid A induced the adventitious root formation of the kidney bean hypocotyls at 1 nM as effectively as indoleacetic acid. Plant growth-promoting properties of endophytic actinobacteria and the recent increased understanding of some of the mechanisms suggest that this promising source merits further investigations for potential application in agriculture.

Facing problems and strategies

The recent advances discussed above have dramatically enlarged our knowledge on the diversity and biotechnological potential of plant-associated endophytic actinobacteria. However, many questions regarding to the ecological function of actinobacteria in the endophytic environments

as well as their evolution and biogeographic distribution remain to be answered. Sustainable exploration of biotechnological potentials of these microorganisms has been limited due to the re-isolation of known strains and products, and many problems still exist in the following areas, needed to be resolved.

Firstly, what is the ‘real’ endophytic actinobacteria? Did endophytic actinobacteria evolve from free-living counterparts (terrestrial soil or even marine microorganisms) through gene loss and acquisition? What are the specific genotypes that make up the endophytic obligate phenotype, or are there specific endophytic actinobacterial genetic markers that will aid in the definition of endophytic actinobacteria? Do endophytic actinobacteria produce plant derived and other compounds that are not found in terrestrial relatives, or what’s difference between them? The endophyte niche is a hot spot for horizontal gene transfer. At present, the HGT hypothesis gives us a plausible alternative postulating the endophyte-host co-evolution. The release of genomes from endophytes will enable comparative genomics studies to better address questions concerning the evolution and ecology basis of endophytic actinobacteria. For instance, Krause et al. (2006) reported the first full genome sequence of an endophyte, strain BH72 of *Azoarcus* species, and this sequence provided valuable insights into the life of bacterial endophytes, including information about interactions with host plants. From the new study of Wu et al. (2010), comparative genomics provided a powerful tool to gain new insights into the niche adaptation of different species of *Pseudomonas putida* (from endophytic and soil) to specific lifestyles and environmental niches, and clearly demonstrated that horizontal gene transfer played a key role in this adaptation process, as many of the niche-specific functions were found to be encoded on clearly defined genomic islands. Clarification and answer of the above-listed issues will demask the real endophytic actinobacteria and guide future rational search and discovery programmes.

Secondly, although collection plants is relatively inexpensive and easy, little is known about the endophytic actinobacterial distribution and abundance compared to soil actinomycetes, and the vast majority of endophytic actinobacteria remain unexplored and unknown. How to isolate more endophytic actinobacterial strains, especially rare actinobacteria and new species? To solve this problem, new isolation strategies and media must be introduced. Four cultivation principles and methods merit particular attention: making endophytes complete release from inner tissues of plant samples and cells enrichment; designing media through simulating host plant nature environments; low nutrient concentration; and extended incubation times. Among the vast plant kingdom, only a handful of plants have been studied. In addition to the tropical rain forests,

those plants growing in harsh habitats such as hot and cold deserts, saline and acidic soils and marine habitats, including algae, bryophytes and lichens should be isolated and screened for bioactive metabolite producing endophytic actinobacteria (Suryanarayanan et al. 2009). New isolation procedures and special habitats plants open a possibility of isolation of new and potential products and processes from them. An issue of concern, which has not been addressed in this review is the Endophytic actinobacterial biogeography. Do endophytic actinobacterial biogeographical distribution and spatial variation reflects the proposition that “the environmental selects”? Understanding the biogeography is not simple of academic interest but also provides a actinobacterial map for biodiscovery.

Third, vast number of uncultured endophytic actinobacteria should not be overlooked. How to cultivate and utilize these microorganisms? Can we find new compounds and genes from uncultured endophytic actinobacteria? The combination of both cultivation-dependent and cultivation-independent techniques in the same study is recommended because cultivation-based techniques enable the recovery and testing of isolates, whereas cultivation-independent techniques enable the screening for variations in the total endophytic communities (Van Overbeek and Van Elsas 2008). The information obtained from culture-independent studies will not only disclose the presence and distribution of endophytic actinobacteria, but also could be used to design selective isolation schemes to cultivate more endophytic actinobacteria, including novel taxa from the same samples. Culturing the previously uncultured endophytic actinobacteria should use some new approaches, such as placing cells in chambers that allow diffusion of compounds from the natural environment, traps enclosed with porous membranes that specifically capture hyphae-forming actinomycetes and growth in the presence of cultivable helper species (Lewis et al. 2010), and this would represent a unique and promising source for the discovery of novel secondary metabolites. Metagenomics and combined high-throughput screening of total DNA from environmental samples also provides an alternative way of discovering new antibiotics and biosynthetic genes. The first metagenomic fosmid library of endophytes was successfully constructed by Wang et al. (2008), which had 1.37×10^6 clones. For example, new gene clusters such as polyketides synthases (PKS) and nonribosomal peptide synthases (NRPS) found in the library indicate the likelihood of novel compounds being produced. This work paved the way for recovery and biochemical characterization of endophytic actinobacterial functional gene repertoire.

Finally, how to quickly find new bioactive secondary metabolites from numerous endophytic actinobacteria? Actinobacterial taxonomic diversity can be used as a

surrogate for chemical diversity. As reviewed by Goodfellow and Fiedler (2010), application of a bioprospecting strategy that a combination of selective isolation, strain dereplication and screening procedures can lead to the discovery of new natural products from novel actinomycetes isolated from geographically diverse samples. As complete sequences of many biosynthetic gene clusters related to different kinds of antibiotics produced by actinobacteria have been gained, it is possible to quickly screen strains that have genes involved in the synthesis of secondary metabolites (Ayuso-Sacido and Genilloud 2004). Additionally, the phylogenetic analysis of the amplified synthase genes can help us to make prediction about the strains that could biosynthesize new compounds with related structural characters. This would open up the possibility of using high-throughput PCR screening method to detect the antibiotic produced endophytic actinobacterial strains. For example, by combined bioactive assay screening, two endophytic *Streptomyces* strains isolated from maytansinoids producer *Trewia nudiflora* Linn were proved to have the potential of producing ansamycins, which further enhanced the hypothesis that endophytes might be involved in the biosynthesis of plant maytansinoids (Zhu et al. 2009). In fact, many other genetic manipulation techniques applied for soil or marine actinomycetes are also available. For example, reengineering artificial or hybrid PKS_S biosynthetic pathways to produce new chemical entities is a reality. Natural gene shuffling, combinatorial biosynthesis, transcriptome analysis and heterologous expression, coupled with recent achievements of genomic sciences, would accelerate the novel natural products discovery from endophytic actinobacteria (Moore et al. 2005; Baltz 2008).

Concluding remarks and perspectives

The past decade have seen a dramatically increase of information in the field of novel species and bioactive compounds isolated from plant associated endophytes. It is therefore necessary to timely review the past successes of endophytic actinobacterial born natural products discovery and to examine future prospects to further explore the biodiversity of endophytic actinobacteria and their produced bioactive secondary metabolites for biotechnological applications.

As reviewed above, the endophytic environment, without any doubt, is keeping a myriad of new actinobacteria providing novel structural diversity to be discovered and used in pharmaceutical, agriculture, and industry. Even so, the study on endophytic actinobacteria is just beginning. We are in the early stages of a renaissance in natural product discovery from endophytic actinobacteria. Future

success not only relies on continued investigations of endophytic actinobacterial resources from wide habitats, but also depends on applied new technologies. Recent applied metagenomic approaches, genome scanning and whole genome sequence mining (Hornung et al. 2007) provide efficient ways for screening gene cluster involved in the biosynthesis of pharmaceutical metabolites and revelation of biosynthesis mechanisms from undiscovered endophytic actinobacteria. Advances in metabolic engineering, combinatorial biosynthesis and heterologous expression will lead to the discovery of new pharmaceutical compounds from endophytic actinobacteria, hopefully with improved therapeutic properties. More successes of whole-genome sequencing and proteomic studies of key endophytes will enhance our understanding of the complicated plant-endophyte interactions and mechanisms and enable better biotechnological applications in plant biocontrol, growth promoting and other areas. We also suggest comprehensive cooperation among global taxonomists, ecologists, natural product chemists, agronomists and bioengineers to better exploit their biodiversity and biotechnological potential.

Acknowledgements This work was partially supported by the National Basic Research Program of China (No. 2010CB833800), National Natural Science Foundation of China (Project no. 30872028, 31000005), the Major Fundamental Research Program of Natural Science Foundation of the Jiangsu Higher Education Institutions of China (08KJA350001), the Program of the Demonstration and Study of Standardization Seeding Technology of *Jatropha* (2007BAD50B0204) and Grants from Natural Science Foundation by Xuzhou Normal University (09XLR12, 09XLR19).

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