Biology and chemistry of endophytes†

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This review focuses on new endophyte-related findings in biology and ecology, and also summarises the various metabolites isolated from endophytes.

1 Introduction

The term ‘endophyte’ is an all-encompassing topographical term which includes all organisms that, during a variable period of their life, symptomlessly colonise the living internal tissues of their hosts.1 This definition is broad enough to include virtually any organism residing inside a plant host. Although our knowledge of the ecology, life history and phylogeny of endophytic fungi has accumulated significantly over the past two decades, fundamental questions regarding the evolutionary origin, speciation and ecological role of such endophytes remain to be answered.2

This article is a follow-up to our previous review (Nat. Prod. Rep., 2001, 18, 448–459), and it focuses particularly on new endophyte-related findings in biology and ecology. It also summarises the various metabolites isolated from endophytes and describes these under different compound classes.

2 Biology of endophytes

2.1 Major groups of endophytic microbes

Endophytic microbes fall into several identifiable classes often in relation to their plant organ source, with the major groups as follows: 1) endophytic Clavicipitaceae; 2) fungal endophytes of dicots; 3) endophytic fungi; 4) other systemic fungal endophytes; 5) fungal endophytes of lichens; 6) endophytic fungi of bryophytes and ferns; 7) endophytic fungi of tree bark; 8) fungal endophytes of xylem; 9) fungal endophytes of root; 10) fungal endophytes of galls and cysts; 11) prokaryotic endophytes of plants (includes endophytic bacteria and actinomycetes).1,2

2.2 The origin and evolution of endophytes

Evidence of plant-associated microorganisms found in the fossilised tissues of stems and leaves has revealed that endophyte–host associations may have evolved from the time that higher plants first appeared on the Earth.3,4 The symbiosis of fungi with plants most probably dates back to the emergence of vascular plants.5 Carroll6 has suggested that some phytopathogens in the environment are related to endophytes and have an endophytic origin. In certain environments, some microbes appear actively to penetrate plant tissues through invading openings or wounds, as well as proactively using hydrolytic enzymes such as cellulase and pectinase. Some bacterial endophytes are believed to originate from the rhizosphere or phylloplane microflora,7 through penetrating and colonising root tissue as an access point to the xylem.8

Majewska-Sawkaa and Gentile9 have traced the presence and distribution of Neotyphodium lolii within developing inflorescences and embryos of perennial ryegrass (Lolium perenne L.), cultivar ‘Grassland Nui’, by in situ immunolocalisation of fungal proteins. Evidence is presented that the fungus penetrates through the rachilla at the base of the ovary, and localises in a very precise and specific manner in the ovular nucellus, but never enters the embryo sac or the integuments. Young embryos do not contain mycelium, but as they mature the hyphae penetrate through the scutellum from a neighbouring ‘infection layer’—a remnant of nucellus heavily colonised by the fungus—that is readily visible as a discrete area directly adjoining the base of embryo cavity. It was shown that N. lolii was transmitted to the embryo exclusively via sporophytic maternal tissue. In vascular tissue, bacteria can travel throughout the host plant and hence colonise it systemically.7
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In control experiments, grazing herbivores reduced the exposed biomass of non-host plants relative to the endophyte host fescue grass. These results demonstrate that herbivores can drive plant–microbe dynamics and, in so doing, they can modify plant community structures either directly or indirectly. In hereditary symbioses, genomes of both partners are co-inherited. Therefore, these symbionts are linked directly to evolutionary changes in their host populations. Hereditary symbionts are transmitted across generations through eggs, seeds, or clonal propagules, and rarely through sperm. They include a diversity of interactions and are especially well known in arthropods and their obligate associations with vertically transmitted bacteria.

During the long co-evolution of endophytes and their host plants, endophytes have adapted themselves to their special microenvironments by genetic variation, including uptake of some plant DNA into their own genomes. This could have led to the ability of certain endophytes to biosynthesise some ‘phytochemicals’ originally associated with the host plants. Fungal endophytes have evolved two transmission modes. These are vertical and horizontal transmission, of which the former transmits the systemic fungus from plant to offspring via host seeds, and the latter operates by sexual or asexual spore transfer. In a co-evolutionary view, endophytic microbes improve the resistance of the host plants to adversity by the secretion of bioactive secondary metabolites. Of course, the evolved relationships between endophytes and their hosts are complex, and involve multi-species interactions, multiple levels of causation and multidirectional flows of influence. Such interactions are affected by stochastic events, such as abiotic and biotic challenges. Dong’s result suggested that endophytic colonisation involves an activation process governed by genetic determinants from both partners. Gentile et al. investigated the phylogenies of 27 Neotyphodium spp. isolates from 10 native grass species in 22 populations throughout Argentina. The evolutionary relationships among these fungi and a worldwide collection of Epichloë endophytes were estimated by phylogenetic analysis of sequences from variable portions (mainly introns) of genes for β-tubulin (tub2) and the translation elongation factor 1-α (tef1). The results showed that most of the Argentine endophyte isolates were interspecific hybrids of Epichloë festucae and E. typhina. Only one isolate was a hybrid of different ancestry, and three isolates were apparently non-hybrid endophytes. Interspecific hybridisation promotes genetic variation, and was clearly a persistent trait during the evolution of endophyte colonisation of Argentine grasses. However, Brem reported that isolates inhabiting B. benekii and B. ramosus represent long-standing host races or incipient species that emerged after host shifts, and that they may have evolved through host-mediated reproductive isolation toward independent species. Moreover, attention to the widespread occurrence of interspecific hybrid Neotyphodium lineages, on a global scale, and the extent of
endophyte gene-flow between the Northern and Southern Hemispheres demonstrated that the Southern Hemisphere endophytes have one lineage of apparently non-hybrid evolutionary origin and three lineages of unique interspecific hybrid evolutionary origin.\textsuperscript{17} N. tembladera\textsuperscript{e} appeared to be of hybrid origin, involving \textit{E. festucae} and an \textit{E. typhina} genotype similar to that of isolates from \textit{Poa nemoralis}.

\subsection*{2.3 Host range}

Fungal surveys of various hosts during the past 20 years have demonstrated that the colonisation of land plants by endophytes is ubiquitous. Endophytes are detected in plants growing in tropical, temperate, and boreal forests with the hosts ranging from herbaceous plants in various habitats including extreme Arctic, alpine\textsuperscript{20} and xeric environments\textsuperscript{29} to mesic temperate and tropical forests. Endophytic fungi have been found in mosses and hepatics,\textsuperscript{26} ferns and fern allies,\textsuperscript{25} numerous angiosperms and gymnosperms including tropical palms,\textsuperscript{22,23} broad-leaved trees,\textsuperscript{34} estuarine plants,\textsuperscript{25,26} diverse herbaceous annuals, and many deciduous and evergreen perennials.\textsuperscript{27,28}

There are approximately 250 000 different plant species on our planet. An estimate of 1 million endophytes seems reasonable if each individual higher plant hosts an average of four endophytes.\textsuperscript{29} In the past century, however, only about 100 000 fungal species including endophytic fungus were described, with an estimated 900 000 fungi still unknown.\textsuperscript{30} Because numerous new endophytic species may exist in plants, it follows that endophytic microorganisms are important components of microbial biodiversity.\textsuperscript{13}

For example, 21 cacti species occurring in various localities within Arizona have been screened for the presence of fungal endophytes, and 900 endophytic isolates belonging to 22 fungal species were isolated. \textit{Cylindropuntia fulgida} possessed the largest endophyte species diversity, while \textit{C. ramosissima} harboured the most endophyte isolates. \textit{Alternaria sp.}, \textit{Aureobasidium pullulans}, and \textit{Phoma} spp. were isolated from several cactus species. The diversity of the endophyte population was low, and no specific host–guest relationships were observed. However, the frequencies of colonisation of the few endophyte species that were recovered was high and was comparable to that reported for tropical plant hosts.\textsuperscript{32}

Endophytes are found in a wide variety of plant tissue types, such as seeds and ovules,\textsuperscript{33} fruits,\textsuperscript{34} stems,\textsuperscript{35} roots,\textsuperscript{36} leaves,\textsuperscript{37} tubers,\textsuperscript{38} buds,\textsuperscript{39} xylem,\textsuperscript{40} rachis\textsuperscript{41} and bark.\textsuperscript{42} It is now widely accepted that endophyte-free plants are few, and this is especially true for shrubs and trees.\textsuperscript{43} Several studies have shown the presence of fungal endophytes in host species belonging virtually to all plant divisions, from mosses and ferns to monocotyledons. The reported data suggests that both bacteria and fungi are the most common endophytic microorganisms.\textsuperscript{44,45}

Endophytic bacteria reside in plant tissues mainly in intercellular rather than intracellular spaces, and inside vascular tissues.\textsuperscript{46} A survey of endophytic bacteria that colonise the roots of carrots (\textit{Daucus carota}) in Nova Scotia has been carried out. Among the 360 isolates examined, 28 bacterial genera were identified, of which \textit{Pseudomonas}, \textit{Staphylococcus}, and \textit{Agrobacterium} were the most common, constituting 31, 7 and 7% of the microbiological populations, respectively. Diversity indices showed no significant differences between the two separate locations.\textsuperscript{47} Endophytic fungi are known to infect hundreds of grasses worldwide. For example, \textit{Epichloë} endophytes are a group of clavicipitaceous fungi (Clavicipitaceae) that form symbiotic associations with a broad range of grasses within the Pooidae subfamily, and they have been the widely studied. Sexual \textit{Epichloë} endophytes behave as mutualists during the vegetative phase of plant growth and systemically colonise the intercellular spaces of leaf primordia, leaf sheaths and culms of vegetative tissue.\textsuperscript{48} Asexual anamorphic \textit{Neotyphodium} species form asymptomatic mutualistic associations with their hosts, and are vertically transmitted through the seed following colonisation of the developing ovule.\textsuperscript{49} Twenty-two collections of \textit{Taxus baccata} and one of \textit{T. brevifolia} were sampled in different habitats located in central to northern Italy, and a total of 150 fungal and 71 actinomycete strains were isolated from the woody and herbaceous tissues.\textsuperscript{50} This was the first report demonstrating the presence of actinomycetes inside living tissues of above-ground organs of plants.

\subsection*{2.4 Host and tissue specificity}

It is possible to isolate hundreds of endophytic species from a single plant, and among them, at least one generally shows host specificity.\textsuperscript{51} Often a single woody plant will harbour more than 40 fungal endophytes.\textsuperscript{51–52} Systemic grass endophytes are shown to be significantly more host-specific than fungal and plant phylogenies, leading to host-adapted fungi that are compatible with only certain host genotypes.\textsuperscript{53} In general, endophytic fungal communities demonstrate single host specificity at the plant species level, but this specificity can be influenced by environmental conditions.\textsuperscript{51} With the exception of \textit{Epichloë typhina}, which has a very broad host range, all other species of the \textit{Epichloë} genus are relatively host-specific.\textsuperscript{54} Investigations on the endophytic community in \textit{Quercus ilex} has revealed a higher degree of single host specificity within the plant’s native geographic range.\textsuperscript{54} Endophytes are also able to colonise multiple host species belonging to different families within a given geographic site. For instance, dark septate root endophytes (DSE) are conidial or sterile fungi that colonise plant roots. They have been reported for nearly 600 plant species, representing about 320 genera and 100 families.\textsuperscript{55} Examination of folioose and crustose algae has revealed a wide range of alternative hosts for \textit{Aerosilpha sporophytes}.\textsuperscript{56} \textit{Phialocephala fortinii} is a common root endophytic fungus with a wide geographic distribution which occurs in both xeric and hydric sites.\textsuperscript{57}

Endophytic fungi also exhibit organ and tissue specificity as a result of their adaptation to different physiological conditions in plants.\textsuperscript{41} Fluctuations in the bacterial profile were determined by different parameters (seasonal changes, plant organs, presence of phytoplasmas), revealing influences such as temperature (warm or cold according to the season) and in the organs examined (e.g. roots or stems).\textsuperscript{58} In addition, more stressful environments drive the selection toward higher infection frequencies of endophytes in grasses.\textsuperscript{59} For instance, summer drought exerts a selection pressure on grass in favour of endophyte infection.\textsuperscript{60,61} Dong \textit{et al.}\textsuperscript{14} assessed the host range and strain specificity for endophytic colonisation with \textit{Klebsiella pneumoniae} 342 (Kp342) on five host plants, in which Kp342 was the most efficient coloniser of the plant apoplast. The monocots inoculated in this study were colonised endophytically in much higher numbers than the dicots. Cells of Kp342 congregate at lateral root junctions, suggesting that the cells enter the plant through cracks created by lateral root
Extensions. *Lasiodiplodia theobromae* is a cosmopolitan fungus with a worldwide distribution in the tropics and subtropics. A study of the genetic diversity and gene flow between populations of *L. theobromae* suggests predominant clonal reproduction with some genotypes widely distributed within a region.62

### 2.5 Isolation

It is important to establish a specific protocol for the isolation of endophytes from a given plant, particularly as 90–99% of microorganisms are not readily cultivable.63 The most frequently isolated microorganisms are endophytic fungi. Scrutiny of the literature shows that there is little difference in the frequency of success of various isolation protocols, and that ‘standardised’ procedures inevitably fail to result in the isolation of certain special endophyte(s).

#### 2.5.1 Methods for the isolation of endophytes

The method most frequently utilised to detect and quantify endophytic fungi involves isolation from surface-sterilised host plant tissue. For reports profiling inventories of endophyte species occurrence and diversity, this is currently the most practiced approach, although fungal biologists recognise that some endophyte groups may be undetected or under-represented, and in particular, isolates that are unable to sporulate in culture may need to be evaluated by other means.6 Host species, sampling strategy, host–endophyte and inter-endophyte interactions, tissue types and ages, geographic and habitat distributions, types of fungal colonisation, culture conditions, surface sterilants, and selective media all influence the detection and enumeration of endophytic fungi.6 Techniques used for isolation, maintenance, identification, and preservation of grass endophytes have been reviewed by Bacon and White.64 Detailed practical information on methods for the isolation of filamentous fungi from various substrata, including techniques, selective agents, and the most common media, is available in the literature.65,66

Surface sterilisation of plant material is usually accomplished by treatment with a strong oxidant or general disinfectant for a period, followed by a sterile rinse. Household bleach (NaOCl), usually diluted in water to concentrations of 2–10%, is the most commonly used surface sterilant. Similarly effective oxidants include 3% H2O2; and 2% KMnO4. Furthermore, the efficacy of surface sterilisation can be substantially improved by combining with a wetting agent. This is particularly appropriate for hydrophobic or densely pubescent leaves. Ethanol (70–95%) is the most commonly used wetting agent in this respect, however, it has limited antibiotic activity, and thus is not used alone as a surface disinfectant.64 Other surfactants such as Tween 80 have found use as wetting agents to enhance surface sterilisation of the host plants.5

#### 2.5.2 Isolation procedures

The host plant should be unambiguously identified in any endophyte-related study, and its global position, as defined by location, latitude, longitude and altitude, should be recorded.2 Endophytes are generally isolated after cutting individual plant organs into segments (3–5 mm long) followed immediately by treatment with bleach.67 Alternatively, plant material is surface-treated with 70% ethanol and then dried under a laminar flow hood.68 Two to three tissue segments are removed every 2–3 minutes and vigorously rinsed in sterile distilled water. These pieces then are pressed into potato dextrose agar (PDA), and the plates are incubated at room temperature for 3–4 weeks. Rapidly growing fungi that appear within the first 2 weeks are generally discarded since they are most probably contaminants. After 2–4 weeks, white to off-white colonies of endophytes become visible. Plates are prepared in triplicate to eliminate the possibility of contamination or heterokaryosis.69 It is advisable to remove outer tissues with a sterile knife blade, and the newly formed surfaces are placed carefully onto agar plates or PDA medium co-supplemented with 200 μg mL−1 ampicillin and 200 μg mL−1 streptomycin to suppress bacterial growth until the mycelium or colony originating from the segments appears.68,70 After several days of incubation, hyphal tips of the fungal endophyte are removed and transferred to newly prepared PDA plates. Some bacterial species such as *Streptomycetes* spp. can survive this treatment. For identification purposes, the endophytes are trained to sporulate on pre-treated plant materials.2

In order to isolate endophytes from plant seeds, the deglume is required to be removed together with contaminants associated with the dry glumes. This is achieved by rubbing the seeds vigorously between the hands and then rinsing the seeds for 15–20 minutes with a bleach solution.2 The isolation of endophytic bacteria is often accomplished by pasting onto LB plates the trituration of plant tissues surface-disinfected with various disinfectants such as sodium hypochlorite, ethanol, hydrogen peroxide, mercuric chloride, or a combination of two or more of these.71

### 2.6 Identification

Rigorous identification of endophytes requires microscopic examination of the host tissue and relies to a significant extent on the taxonomic expertise of the examiner. Morphological examination is performed by scrutinising the culture, the mechanism of spore production, and the characteristics of the spores. This is especially valuable for isolates failing to produce spores or identifiable structures.

Sometimes, optimisation of growth conditions aiming at inducing sporulation of endophytes is a trial-and-error process.2 Each of the isolated fungal strains is separately inoculated on PDA, CMA, CA, WSA and PCA media in Petri dishes to achieve optimum conditions for sporulation.68 Moreover, endophytic fungi that neither grow nor sporulate in culture can only be detected and identified by other means such as a comparison of ribosomal DNA (rDNA) gene sequences, an analysis that can be used to determine phylogenetic relationships.72 Accordingly, endophytic isolates are often identified using a combination of morphological and molecular methods.73,74

Special caution has to be taken when closely related or morphologically similar endophytic fungi are under identification. The morphological features of some fungi are usually medium-dependent, and some cultural conditions can affect substantially vegetative and sexual incompatibility. Thus, the morphological character of endophytes should be coupled with the available molecular evidence to enable significant differentiation between closely related species. For newly discovered endophytic fungi, morphology-based identification is confirmed by 18S rDNA sequence comparisons or internal transcribed spacer (ITS1 and ITS2) and 5.8S rDNA sequence examinations. For instance, the producing strain of hormonemate was identified unambiguously
as *Hormonema dematioides* by microscopy and ITS rDNA sequence analysis. An endophytic isolate from the roots of vascular plants was characterised on the basis of cultural and morphological properties and PCR/RFLP analysis of the ITS region and a portion of the 28S subunit of rDNA. The restriction digest profiles of all isolates were identical to those of *Phialocephala fortinii* for four restriction enzymes, and DNA sequences showed a low percent sequence divergence, confirming the reliability of the RFLP data.

Bacterial endophytic microbes are analysed by 16S rRNA-based techniques. However, several biomolecular methods generally combined to identify unknown endophytic isolates. As an example, 16S rDNA cloning and sequencing, terminal restriction fragment length polymorphism (T-RFLP) analysis and denaturing or temperature gradient gel electrophoresis (D/TGGE) were combined to study diversity of bacterial endophytic populations in potato cultivars. Molecular techniques, as a powerful tool, are used to identify endophytic genera and species and explore the unique trophic niches occupied by non-sporeulating and unculturable fungi. However, the use of molecular databanks, such as GenBank, for species identification is limited in several regards. It may be accepted as a reliable molecular technique once molecular databanks accurately reflect species collected from a broad range of geographic areas/environments.

### 2.7 Physiological role

It is generally accepted that endophytic microbial communities play an important beneficial role in the physiology of host plants. Plants infected with endophytes are often healthier than endophyte-free ones. This effect may be partly due to the endophytes' production of phytohormones (such as indole-3-acetic acid (IAA), cytokines, and other plant growth-promoting substances like vitamins) and/or partly owing to the fact that endophytes can enhance the hosts' absorption of nutritional elements such as nitrogen and phosphorus and that they regulate nutritional qualities such as the carbon–nitrogen ratio. For example, roots of *Populus* *Esh5* explants were inoculated with *Piriformospora indica*, and there was an increase in root biomass, with the number of 2nd-order roots increasing significantly. However, Bonnet et al. found that selected strains of bacterial endophytes from the carrots in the potato bioassay had differential effects on plant growth. It emerged that 38% of the endophytes remained growth-neutral, 33% promoted and 29% inhibited plant growth.

Protective effects on endophyte-infected host plants greatly enhance their resistance to unfavourable challenges. The evidence suggests that plants infected with endophytic fungi often have a distinct advantage against biotic and abiotic stress over their endophyte-free counterparts. Beneficial features have been offered in infected plants, including drought acclimatisation, improved resistance to insect pests and herbivores, increased competitiveness, enhanced tolerance to stressful factors such as heavy metal presence, low pH, high salinity, and microbial infections. Endophyte-infected plants also gain protection from herbivores and pathogens due to the bioactive secondary metabolites that endophytes generate in plant tissue. An increasing number of antimicrobial metabolites biosynthesised by endophytic microorganisms, such as alkaloidal mycotoxins and antibiotics, have been been detected and isolated.

The endophytic strain *P. indica* is reported to induce resistance to fungal disease and tolerance to salt stress in barley, a monocotyledonous plant. A beneficial effect on the defence status of the plant was detected in distal leaves, demonstrating a systemic induction of resistance by a root-endophytic fungus. The systemically altered ‘defence readiness’ was associated with an elevated antioxidative capacity due to activation of the glutathione–ascorbate cycle, and this resulted in an overall increase in grain yield. It has been shown that symbionts mediate resistance to parasites in *Acrystosiphon pisum*, rather than aphid genotype. In a controlled genetic background, it has been shown that the symbiont confers resistance to parasite attack by inhibiting the development of the parasite larvae. Arnold et al. found that endophyte-mediated protection was primarily localised to endophyte-infected tissues. Furthermore, the protection was greater in mature leaves, which bear less intrinsic defence against fungal pathogens than do the young leaves.

The role of endophytes in influencing plant physiology has been studied. Some endophyte-infected plants interact directly or indirectly with mineral nutrient uptake to reduce or prevent stress. Drought-tolerant species, endophytic fungi exert their action not only in the storage and secretion of sugars and alcohols, but also in the modification of leaf characteristics, which reduces transpiration losses. Under heavy metal stress, endophytic microbes can protect host plants by limiting heavy metal transport and metal accumulation in plant tissues.

### 2.8 Ecological role

Endophytic microorganisms play an important role in ecological systems through shaping plant communities and mediating ecological interactions. Under ambient mammalian herbivory, the above-ground biomass of non-host plant species was lower than with the mammal exclusion treatment, and plant composition shifted toward greater relative biomass of infected, tall fescue grass. These results demonstrate that herbivores can drive plant–microbe dynamics and modify plant community structure directly and indirectly.

In some plants, endophytic fungi perform novel ecological functions (e.g. thermotolerance of plants growing in geothermal soils). Endophytes can influence community biodiversity, and microbial interactions have been shown to be important determinants of plant biodiversity. In grasses and other herbaceous plants, dominant endophytes are known to produce toxic alkaloids that deter or poison herbivores. In woody plants, endophytes may also function in specific defence roles or more generally act to diminish or avoid pathogen damage. Together with mycorrhizal fungi, endophytes form an integral part of the extended phenotype or symbiotic community of a plant. The full range of ecological functions of the endophytes of woody plants is poorly understood, but it is likely to be correlated with their species diversity. A study of plant diversity in successional fields in the eastern USA showed that the expansion of the association formed by tall fescue and *Neotyphodium coenophialum* reduced plant biodiversity. The reason was ascribed to the high level of alkaloid toxins resulting from this association, which may alter the feeding patterns of small mammalian herbivores, birds and insects, thereby altering the community structure. Fungal endophytes of pasture grasses (mainly *Festuca arundinacea*) were found to...
negatively affect mycorrhizal fungi, and exert significant effects on components of detrital food webs, including earthworms, nematodes, collembolans, mites, and soil microflora.

Grass endophytes had been shown to have a major impact on regulating terrestrial food webs. By altering the litter quality for detritivores and/or the microenvironment for decomposition, foliar endophytes of grasses might influence litter decomposition and carbon and nutrient cycling.

3 Chemistry

Endophytic microorganisms are a significant reservoir of genetic diversity, and an important source for the discovery of novel bioactive secondary metabolites. Endophytes are a rich source of natural products displaying a broad spectrum of biological activities, and the phytochemistry of endophytic microbes continues to increase in significance. As a general rule, a single endophytic strain will produce multiple bioactives. The reported natural products from endophytes includes antibiotics, antipathogens, immunosuppressants, anticancer compounds, antioxidant agents and other biologically active substances. This section describes the functional metabolites from bacterial, actinomycetous and fungal endophytes characterised since 2001.

3.1 Endophytic bacteria

3.1.1 Biomacromolecules. Polysaccharides and enzymes are common macromolecules from bacterial endophytes. Polysaccharides, such as bacterial exopolysaccharides (EPSs) and lipopolysaccharides (LPSs), play an important role in plant–bacteria interactions and colonisation. Two acidic EPSs, EPS A and EPS B, had been isolated from a Burkholderia brasiliensis strain M130, associated with rice roots. The repeat unit of EPS A contained two L-Rha, two D-Glc and a D-GlcA residue, and that of EPS B possessed two L-Rha, two D-Glc, two D-GlcA and two D-Gal residues. LPSs were purified and characterised by denaturing electrophoresis from an endophytic strain of B. cepacia in Asparagus officinalis. These metabolites could induce systemic resistance in host plants against pathogens and suppress the hypersensitive response under certain conditions.

Endophytic bacteria can produce proteins and enzymes with important biological functions. A strain of Bacillus subtilis BS-2 from capsicum leaves was found to produce an antifungal protein, which was thermostable and UV-tolerant. Pleban et al. isolated a chinatinase with a molecular mass of 36 kDa from an endophytic B. cereus present in mustard. The enzyme exhibited stability between pH 4.0 and 8.5, and significantly protected cotton extracellular pectinase from degradation. LPSs were purified and characterised growing in Grevillea pteridifolia.

3.1.2 Secondary metabolites. Secondary metabolites, such as phytohormones and salicylic acid, are frequently isolated from endophytic bacteria. An endophytic fluorescent bacterium Pseudomonas viridiflava associated with leaves of many grass species. A group of novel antifungal lipopeptides named ecomycins, which contain some unusual amino acids such as homoserine and β-hydroxyaspartic acid. Methylobacterium extorquens and Pseudomonas synxantha are two endophytic bacteria from meristemetic bud tissues of the Scots pine (Pinus sylvestris L.). They are found to produce adenine derivatives which may have a role as precursors in cytokinin biosynthesis.

3.2 Endophytic actinomycetes

3.2.1 Biomacromolecules. It is generally accepted that the actinomycetes (especially streptomycetes) have a particular capacity to elaborate antibiotics. Ever since Gurney and Mantle isolated 1-N-methylallothronour from an Acremonium-like Streptomyces sp. living in perennial ryegrass seed tissue, a broader search for endophytic actinomycetes, and particularly the identification of bioactive macromolecules, has been carried out. For example, a strain of Nocardiosis sp. was isolated from yam bean (Pachyrhizus erosus L. Urban) and found to excrete an α-amylase with thermostable characteristics, as indicated by the retention of 100% of residual activity at 70 °C and 50% of residual activity at 90 °C for 10 min.

3.2.2 Secondary metabolites. Castillo et al. have isolated an endophytic Streptomyces sp. NRRL 30562 from the snakevine, which produced four novel antibiotics, munumbicins A, B, C and D with masses of 1269.6, 1298.5, 1312.5 and 1326.5 Da, respectively. These metabolites displayed broad-spectrum activity against pathogenic fungi and bacteria. From the cultures of streptomycete NRRL 30566 of Grevillea pierdiophila growing in the Northern Territory of Australia, several kadayumycins were purified and characterised, each of them containing alanine and serine residues as well as an unknown amino acid. Biological assays indicated that kadayumycin A had the more potent activity than echinomycin against B. anthracis and the malarial parasite Plasmodium falciparum, with minimum inhibitory concentrations of 0.2–0.3 μg mL⁻¹, and LD₅₀ values of 7–10 ng mL⁻¹, respectively. In addition, kadayumycin A exhibited inhibitory effects on RNA synthesis. The coronamycins, a complex of novel peptides, was isolated from a verticillate Streptomyces sp. in Monstera sp., and displayed bioactivities against pythiaceous fungi, the human fungal pathogen Cryptococcus neoformans, and the malarial parasite, Plasmodium.

Two new germacrine-type sesquiterpenes, 1(10),5E-germacadiene-3,11-diol 1 and 1(10),5E-germacadiene-2,11-diol 2, were purified together with 1(10),5E-germacadiene-11-diol 3 from a Streptomyces griseus subsp. colonising the mangrove plant Kandelia candel. More recently, Lin et al. isolated four novel cyclopentenone derivatives 4–7 from an unidentified Streptomyces sp. endophytic on the mangrove plant Aegiceras corniculatum collected at the coastline near to Xiamen, in the Fujian Province of China.
3.3 Endophytic fungi

3.3.1 Biomacromolecules. Fungal endophytes are seldom reported to produce polysaccharides, enzymes or proteins, in contrast to other endophytes. A mycelium-bound lipase isolated from *Rhizopus oryzae*, an endophyte of the Mediterranean plant *Foeniculum vulgare* (fennel), was shown to be active over the pH range 3–8, and was thermostable, with maximal activity at 60 °C. A novel glucoamylase with a strong amylopectin-hydrolysing activity was found in the culture filtrate of endophytic *Acremonium* sp., and this enzyme exhibited biological stability between pH 3.0–7.0 and also up to 60 °C.

3.3.2 Secondary metabolites. The number of secondary metabolites produced by fungal endophytes is larger than that of any other endophytic microorganism class. This may of course be a consequence of the high frequency of isolation of fungal endophytes from plants. Natural products from fungal endophytes have a broad spectrum of biological activity, and can be grouped into several categories, including alkaloids, steroids, terpenoids, isocoumarins, quinones, phenylpropanoids and lignans, phenol and phenolic acids, aliphatic metabolites, lactones, etc.

(a) Alkaloids. Most of the alkaloids have been detected in the cultures of grass-associated endophytic fungi, such as sexual *Epichloë* spp. and asexual *Neotyphodium* spp. These metabolites play an important role in inhibiting herbivores and insects. Although the alkaloid levels may be influenced by environmental factors, the types and levels of alkaloids seem to depend mostly on endophyte species, strain, or genotype and less on the host grass genotype and the environment. The alkaloids from fungal endophytes include amines and amides, indole derivatives, pyrrolizidines and quinazolines.

Amines and amides. Amines and amides are common substances produced by fungal endophytes from tall fescue, perennial ryegrass and many temperate grasses. Peramine 8 is a pyrrolopyrazine alkaloid, and its biosynthetic pathway has been proposed as illustrated in Scheme 1. The production of this metabolite was significantly affected by the host plant genotype, rather than the endophytic haplotype or environmental factors. Ergot alkaloids are usually detected in the cultures of endophytic fungi belonging to *Neotyphadium* spp. and *Epichloë* spp. found in several grass species.

The three amides 9–11 were characterised as decalin tetramic acid type antibiotics. Compound 9 from an endophytic *Phoma* sp. was shown to inhibit ras-farnesyltransferase. Cryptocin 10, a potent antymycotic against *Pyricularia oryzae* and other phytopathogens, was elaborated by *Cryptosporiopsis cf. quercina* present in the bark of the stems of *Tripterygium wilfordii*. Metabolite 11, together with tenuazonic acid 12, was purified from the culture broth of two endophytic *Alternaria* spp. P0506 and P0535, and displayed potent activity against pathogenic Gram-positive bacteria. Four botryane-type metabolites, L-696474 13, cytochalasin U 14, RKS-1778 15 and cytochalasin H 16, were isolated from *Geniculosporium* sp. 6580, an endophytic fungus from the red alga *Polysiphonia* sp. Structurally, these cytochalasins are composed of a highly substituted isodolone ring with a benzyl group at the C-3 position. Peniprequinolone 17, gliovictin 18 and gliovictin acetate 19 were metabolised by *Penicillium janczewskii* K. M. Zalesky of the Chilean gymnosperm *Pruinopitys andina*. Three new p-aminoacetophenonic acids 20–22 from the mangrove fungal endophyte *Streptomycetes griseus* subsp., seem to be precursors of the aminoacetophenone heptaene antibiotics, such as levorin and trichomyacin.

In our laboratory, three novel alkaloids, asperflumod 23, aspernigrin A 24 and aspernergirin 25, were isolated from *Aspergillus fumigatus* CY018, *Cladosporium herbarum* IFB-E002 and *Aspergillus niger* IFB-E003 respectively, all endophytes of *Cynodon dactylon*. Bioactivity tests indicated that compound 24 inhibited *Candida albicans* with an MIC of 75.0 μg mL⁻¹, and that 25 had moderate cytotoxic activity against tumour cells (nasopharyngeal epidermoid KB), cervical carcinoma Hela and human colorectal carcinoma SW1116, with corresponding
IC$_{50}$ values of 22, 46 and 35 $\mu$M, respectively. The chemical synthesis of aspernigerin 25 was achieved in 25% overall yield in four steps, as illustrated in Scheme 2. Two cerebrosides, 26 and 27, with antibacterial and xanthine oxidase inhibitory activities were identified from an endophytic Fusarium sp. IFB-121 in Quercus variabilis. Additionally, two novel ceramides 28 and 29 were isolated from an unidentified endophytic fungus (No. 2524) obtained from the seeds of mangrove Avicennia marina.

**Indole derivatives.** The indole derivatives lolitrem C 30 and F 31 have been shown to have neurotoxic activities, and to confer resistance against a number of insect species. McMillan et al. found that paxilline and lolitrem B produced tremors in livestock through inhibiting large conductance calcium-activated potassium channels. Two anticancer indole derivatives, vincristine 32 and chaetoglobosin A 33, have been purified from Fusarium oxysporum in Catharanthus roseus (L.) G. Don and Chaetomium globosum in Maytenus hookeri. More recently a new cytochalasan alkaloid, chaetoglobosin U 34, was separated together with chaetoglosins C 35, E 36, F 37, and penochalasin A 38 from C. globosum IFB-E019 in Imperata cylindrical. All of these compounds exhibited cytotoxic activities against the human
nasopharyngeal epidermoid tumour KB cell lines with IC\textsubscript{50} values of 16, 34, 52, 48, and 40 μM, respectively.

**Pyrrolizidines.** Pyrrolizidines, especially 1-aminopyrrolizidines with an oxygen bridge, are a common metabolite in some grass–endophyte associations. The loline alkaloids are the only grass–endophyte-associated alkaloid class that protects endophyte-infected plants due to their anti-invertebrate and feeding deterrent activities.\textsuperscript{183} A biosynthetic pathway for norloline \textsuperscript{39} has been proposed, as shown in Scheme 3. Incorporation of isotopically labeled L-proline and L-homoserine into \textsuperscript{39} indicated that the A-ring carbons C1–C3 and the N1 are derived from L-homoserine, and that the B-ring carbons C5–C8 and the ring nitrogen are derived from L-proline.\textsuperscript{184}

**Quinazolines.** Structurally, quinazolines correspond to products of condensation of anthranilic acid with α-amino acids.\textsuperscript{185} Four rare spiroquinazoline alkaloids, alanditrypinone \textsuperscript{40}, alantryphene \textsuperscript{41}, alantryphine \textsuperscript{42} and alantryleneunone \textsuperscript{43}, were isolated from the culture of the endophytic fungus \textit{Espericillium} sp. residing in leaves of \textit{Murraya paniculata} (Rutaceae).\textsuperscript{186} These alkaloids seem to be biosynthesised by a unique pathway because their precursors, anthranilic acid and tryptophan, could not be detected in the host plant.

**(b) Steroids.** Steroids, which are extensively distributed in plants, have many important physiological effects, and interestingly some steroidal metabolites from endophytic fungi have also been reported. A novel ergosterol derivative, (20S,22S)-4α-homo-22-hydroxy-4-oxaergasta-7,24(28)-dien-3-one \textsuperscript{44}, was isolated from a strain of \textit{Gliocladium} sp., an endophyte on \textit{Taxus chinensis} (Pilg.) Rehd.\textsuperscript{187} 3β-Hydroxyergosta-4,22-diene \textsuperscript{45} together with ergosterol and 3β-hydroxy-5a,8a-epi-dioxyergosta-6,22-diene
was elaborated by the endophytic *A. fumigatus* CY018 found in *C. dactylon*.

**Terpenoids.** A number of terpenoid derivatives are which produced by fungal endophytes have been reported during 2001–2005. They involve sesquiterpenes and diterpenes, some of which are analogues arising from metabolic degradation of terpenoid skeletons.

**Sesquiterpenes.** In addition to four cytochalasins, eleven novel sesquiterpenoids 46–56 were isolated from cultures of the mitosporic fungus *Geniculosporium* sp., an endophyte associated with the red alga *Polysiphonia* sp. These 11 botryane compounds exhibited moderate inhibitory activity against *Chlorella fusca*, *Bacillus megaterium* and *Microbotryum violaceum*. Macrocyclic trichothecenes are toxic sesquiterpenoids, which can cause serious diseases in livestock, especially during the flowering season.

Six trichothecenes, roridins A 57, D 58, E 59 and H 60, and verrucarins A 61 and J 62, were detected in the culture of an endophytic fungus *Ceratopicnidium baccharidicola* from *Baccharis coridifolia*. The production of these compounds was greater on rice culture than in liquid cultures (YES and MYRO broths). In our laboratory, three novel cytotoxic 10,13-cyclooctatricone-derived macrolides, myrothecines A–C (63–65), were separated from *Myrothecium roridum* IFB-E009 and IFB-E012, endophytes associated with the two traditional Chinese medicinal plants *Trachelospermum jasminoides* and *Artemisia annua*, respectively. The absolute stereochemistry of these macrolides was established by a combination of NMR of a Mosher’s acid derivative followed by single-crystal X-ray diffraction analysis.
**Diterpenes.** Guanacastepenes A–O 66–80, a highly diverse family of diterpenoid natural products, were identified from an unidentified endophytic fungus CR115. By comparison with other guanacastepenes, metabolites 66 and 73 exhibited pronounced antibiotic activity against drug-resistant strains of *Staphylococcus aureus* and *Enterococcus faecalis*. Scheme 4 shows putative biosynthetic relationships between these metabolites. In addition to important ring-generating biosynthetic transformations, some oxidation/reduction and adornment reactions (e.g. methylation and acetylation) are involved in the biosynthesis of the individual guanacatepenes.

**Isocoumarin derivatives.** Over the period 2001–2005, only three novel isocoumarin derivatives, 81–83, have been identified from endophytic sources. These metabolites were isolated from *Geotrichum* sp., an endophyte of *Crassocephalum crepidioides*. Biological assays demonstrated their antimalarial, antituberculous and antifungal activities.

**Quinones.** Two highly functionalised cyclohexenone epoxides, jesterone 84 and hydroxyjesterone 85, were characterised from a newly identified endophyte *Pestalotiopsis jesteri* present in *Fragræa bodenii*. Notably, metabolite 84 displayed selective antifungal activity against phytopathogens. The total synthesis of 84 was accomplished in 14 steps, as illustrated in Scheme 5. The route involved a diastereoselective epoxidation of a chiral quinone monoketal derivative and regio- and stereoselective reduction of a quinone epoxide intermediate. Ambuic acid 86 is a quinone epoxide metabolite with potent antifungal activity from *Pestalotiopsis* spp. and *Monochaetia* sp. living in *Torreya taxifolia*.

*Scheme 4*  Putative biosynthetic relationships within the family of guanacatepenes A–O 66–80.
Jiang et al.\(^{197}\) have isolated three anthracenediones, 87–89, from an unidentified endophytic fungus, no. 1403, colonising mangroves. Xanthoviridicatins E 90 and F 91 are two novel quinone-related metabolites produced by an endophytic *Penicillium chrysogenum* colonising an unidentified plant. These metabolites inhibit the cleavage reaction of HIV-1 integrase with IC\(_{50}\) values of 6 and 5 \(\mu\)M, respectively.\(^{198}\) More recently, seven anthraquinones, 92–98, with potent cytotoxic activities against human colon (SW1116) and leukaemia (K562) cancer cell lines were separated from *Pleospora* sp. IFB-E006 associated with *Imperata cylindrical*.\(^{199}\)

(f) Phenylpropanoids and lignans. Guignardic acid 99, the first member of a novel class of natural products, was detected in the culture broth of *Guignardia* sp. obtained from *Spondias mombin*.\(^{200}\) The oxidative deamination products of L-valine and L-phenylalanine (dimethylpyruvic acid and phenylpyruvic acid respectively) are biogenetic precursors of this metabolite.

(g) Phenols and phenolic acids. Phenols and phenolic acids from fungal endophytes usually have pronounced biological and antioxidant activities. Pestacin 100 and isopestacin 101, two novel dihydroisobenzofuran-carrying phenols possessing antifungal and antioxidant activities, were separated from *Pestalotiopsis microspora* associated with the combretaceaeous plant *Terminalia morobensis* of Papua New Guinea.\(^{202,203}\) Orsellinic acid 105 and the three novel esersglobosumones A–C 102–104 were isolated from *Chaetomium globosum* endophytic on *Ephedra fasciulata* (Mormon tea).\(^{203}\) Compound 102 had a moderate inhibitory effect on the cell proliferation of lung cancer, breast cancer, CNS glioma and pancreatic carcinoma.

(h) Aliphatic compounds. Chaetomenelic acid A 106, a potent and highly specific inhibitor of farnesyl-protein transferase (FP-Tase), was characterised from the endophyte *Chaetomella acetisae*.
Several alternate syntheses of this bioactive natural product had been reported. Notably, a straightforward synthetic pathway was achieved in only two steps with an 89% overall yield (Scheme 6).

**Scheme 6** A facile synthetic synthesis of chaetomellic acid A 106.

*Reagents and conditions: (1) (a) PPh₃, AcOH, CH₃(CH₂)₁₂CHO, reflux, 18 h, (b) 140–150 °C, 30 min; (2) (a) KOH, H₂O–CH₃OH–THF, reflux, 2 h, (b) H⁺/HCl.*

*(i) Lactones.* The seven lactones 107–113, which were originally characterised from a Chilean ascomycete,²⁰⁶,²⁰⁷ were re-detected in an unidentified endophytic fungus associated with *Cistus salviifolius* L.²⁰⁸ Phomol 114 is a novel antibiotic from a *Phomopsis* species present in the medicinal plant *Erythrina crista-galli*.²⁰⁹ Microcarpalide 115, a novel microfilament-disrupting agent with weak cytotoxicity to mammalian cells, was characterised from an unidentified fungus in *Ficus microcarpa* L.²¹⁰ Four total syntheses of compound 115 have been described.²¹¹–²¹⁴ The first convergent and stereoselective synthetic pathway of 115 was achieved by Murga *et al.* starting from (R)-glycidol and (S,S)-tartaric acid, as shown in Scheme 7.²¹¹

Two novel lactones, 1893A 116 and B 117, have been characterised from the extract of the endophyte strain no. 1893 present in an estuarine mangrove on the South China Sea coast.²¹⁵ Sequoiamonascins A–D 118–121 with a novel carbon skeleton, are elaborated by the fungal endophyte *A. parasiticus* and are reported to display moderate activities against cancer cell lines, including MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS).²¹⁶

**Scheme 7** A synthetic pathway to microcarpalide 115.

*Reagents and conditions: (1) (a) TPSCl, Et₃N, DMAP, CH₂Cl₂, rt, 18 h, 93%; (b) CH₃(CH₂)₄MgBr, CuI, THF, −30 °C, 87%; (2) MOMCl, Et₃N, DMAP, CH₂Cl₂, rt, 18 h, 93%; (3) TBAF, THF, 5 h, rt, 93%; (4) (COCl)₂, DMSO, CH₂Cl₂, −78 °C then N,N-diisopropylethylamine, 2 min at −78 °C, then rt; (5) Bu₃SnCH₂CH=CH₂, MgBr₂·Et₂O, 3 Å MS, CH₂Cl₂, 3 h at −78 °C, then 1.5 h at −40 °C, 60% yield over two steps; (6) DCC, DMAP, CH₂Cl₂, rt, 18 h, 86%; (7) 20 mol% catalyst A, CH₂Cl₂, reflux, 24 h, 67%; (8) SMes₂, BF₃·Et₂O, −10 °C, 30 min, 71%; (9) (CH₃SH)₂, BF₃, CH₂Cl₂, 0 °C, 1 h, 66%.²¹¹*
Four 6H-dibenzo[ghi]pyran-6-one derivatives, alternariol monomethyl ether (AME) 122, and graphislactones A, G and H 123–125, were isolated from Cephalosporium acremonium IFB-E007 colonising Trachelospermum jasminoides (Lindl.) Lem in our laboratory. Metabolites 122 and 123 had been isolated from cultured lichen mycobionts of Graphis prunicola, G. cognata and G. scripta. Metabolites 122–125 were shown to be substantially cytotoxic against SW1116 cells with IC50 values of 8.5, 14, 12, and 21 μg mL⁻¹, respectively.

(j) Miscellaneous metabolites. Sequoiatones C–F 126–129 are novel cytotoxic metabolites isolated from Aspergillus parasiticus present in the coast redwood tree Sequoia sempervirens. In addition to asperfunmoid 23, asperfunin 130 was purified from the endophyte A. fumigatus CY018. Rhizotonic acid 131, a novel anti-Helicobacter pylori metabolite, together with monomethylsulochrin 132, was characterised from a Rhizoctonia sp. (Cy064) endophytic on Cynodon dactylon. Four antimicrobial naphtho-γ-pyrones, rubrofasarin B 133, fonsecinone A 134, asperprone B 135 and aurasperone A 136, have been identified in cultures of A. niger IFB-E003 obtained from C. dactylon. Biological assays indicate that compound 133 has potent cytotoxic activity against the colon cancer cell line SW1116 (IC50 4.5 μg mL⁻¹), and compound 136 exhibited inhibition on xanthine oxidase with an IC50 value of 10.9 μM. Compounds 134 and 136 were re-isolated from...
A. aculeatus in Melia zedaardach (Meliaceae). A new xanthene-based metabolite, paranolin 137, was characterised from an endophytic strain of Paraphaeosphaeria nolinae IFB-E011 from Artemisia annua (Asteraceae). Recently, Dai et al. isolated six novel compounds from a Phomopsis sp. endophytic on Adenocarpus foliolosus, which were identified as phomosines D–G 138–141, 6-isopropylcyclohex-1-enecarboxylic 142 and (1aS,3R,4R,4aR,6S,7R,8aS)-7-chloro-3,6-dihydroxy-3,4,8,8-tetramethyloctahydro-1aH-naphtho[1,8]oxirene-4-carboxylic acid 143.

4 Potential applicability

4.1 Plant growth enhancers

It is generally accepted that the plant kingdom is extensively colonised by endophytic microorganisms which form non-pathogenic relationships with their hosts. In addition to protecting plants from biotic and abiotic influences, other nutritional benefits extend from such associations. Although agrochemicals are a major aspect of crop yield improvement they can have environmental problems. Empirical evidence indicates that some endophytic microbes may act as plant fertilisers by enhancing nitrogen fixation and phosphorus assimilation.

Numerous species of endophytic rhizobacteria (PGPR) increase the availability of nutrients in the rhizosphere, with direct benefits to root growth and morphology, and they are beneficial to many aspects of plant–endophyte symbioses. For example, nitrogen-fixing Klebsiella oxytoca VN13 and phosphorus-assimilating Xanthomonas maltophilia VN12 have been co-mixed to form an inoculant ‘Duet’ for seeds. The results indicate that corn inoculated with this ‘Duet’ generates increased yields, and possesses a higher percentage of protein. Similarly, endophytic bacteria in rice (Oryza sativa L.) can effectively colonise host tissues and form nitrogen-fixing symbioses. Such applications of endophytes offers an effective alternative to agrochemicals.

4.2 Phytoprotectors

Endophytes endow their host plants with many benefits, and their commercial potential could reasonably receive more attention. For example, Pantoea agglomerans is an endophyte of crop plants including pea, potato, sweet corn and tomato, and has been shown to effectively control bacterial plant diseases. Heteroconium chaetospira, a root endophytic fungus associated with Chinese cabbage, acts as a control agent against clubroot and Verticillium yellows. Chen et al. found six endophytic strains, Aureobacterium saeraperae, Bacillus pumilus, Phyllobacterium rubiacearum, Pseudomonas putida, P. putida, and Burkholderia solanacearum, which could significantly reduce vascular wilt in cotton caused by Fusarium oxysporum f. sp. vasinfectum. A plant-growth-promoting rhizobacterium, Pseudomonas sp. strain PsJN, has been shown to display an antagonist effect on the in vitro growth and development of Botrytis cinerea, which is a fungal pathogen causing grey mould diseases. It seems that the endophyte inhibits the growth of B. cinerea by disrupting cellular membranes and inducing cell death. An endophytic Streptomyces spp. obtained from tomato (Lycopersicon esculentum), was found to effectively control Rhizoctonia solani, which is one of the most serious and widely spread diseases in tomatoes, sometimes causing more than 70% seedling mortality.

A novel application of endophytic microbes has been explored in the field of phytoremediation to metabolise compounds associated with chemical waste. Certain endophytes act as phytoremediators by degrading compounds which present an environmental hazard. This ‘green’ approach to such management is gaining public attention. For example, the newly identified endophytic bacterium Methylobacterium populum sp. was shown to degrade 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (HMX) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX).

5 Concluding remarks

Ever since penicillin was isolated from Penicillium notatum, chemists have been engaged in the discovery of novel bioactives from microbial metabolites. Despite a focused interest on synthetic products, bioactive natural products retain an immense impact on modern medicine. Around 60% of the new drugs registered during the period 1981–2002 by the FDA as anticancer, antimigraine and antihypertensive agents are either natural products or based on natural products. Endophytic microorganisms have developed the biochemical ability to produce compounds similar or identical to those produced by their host plants as a result of gene recombination during the evolutionary process. Bioactive natural products from endophytic microbes have enormous potential as the source of new medicinal and agricultural products, and methods to facilitate the identity of appropriate natural products from this source are required. This aspect adds further weight to the preservation of plant biodiversity and greater organisation in the collection and cataloguing of endophytic microorganisms throughout the world.

There has been an improved understanding of biosynthetic pathways to some bioactive endophytic compounds by chemical and biochemical means, and recent progress in the molecular biology of secondary metabolites offers a better insight into how the genes for these bioactive compounds are organised. As a relatively poorly investigated group of microorganisms, the relationship between endophytes and their hosts merits improved quantitative analysis, particularly at the molecular and genetic levels. The cloning of the genes of endophytic metabolites has begun to open up attractive screening possibilities for the direct identification of endophytic strains.
6 References


