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Bacterial diterpene synthases: new opportunities for mechanistic enzymology and engineered biosynthesis

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Diterpenoid biosynthesis has been extensively studied in plants and fungi, yet cloning and engineering diterpenoid pathways in these organisms remain challenging. Bacteria are emerging as prolific producers of diterpenoid natural products, and bacterial diterpene synthases are poised to make significant contributions to our understanding of terpenoid biosynthesis. Here we will first survey diterpenoid natural products of bacterial origin and briefly review their biosynthesis with emphasis on diterpene synthases (DTSs) that channel geranylgeranyl diphosphate to various diterpenoid scaffolds. We will then highlight differences of DTSs of bacterial and higher organism origins and discuss the challenges in discovering novel bacterial DTSs. We will conclude by discussing new opportunities for DTS mechanistic enzymology and applications of bacterial DTS in biocatalysis and metabolic pathway engineering.

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Introduction

Terpenoids comprise the largest, structurally most diverse family of natural products and play important roles in all living organisms. Among the ~60 000 members known to date, ~12 000 are diterpenoids, most of which are produced in plants and fungi. Diterpenoids of bacterial origin are known but rare, however recent advances in genomics have revealed that the biosynthetic potential for terpenoids in bacteria, particularly in the actinomycetes, may be significantly underestimated [1,2].

Diterpenoid biosynthesis has been extensively studied in plants and fungi [3–6], yet cloning the respective genes and characterizing and engineering diterpenoid pathways in these higher organisms remain challenging [7,8]. Scattering of the biosynthetic genes on the genomic DNA of these higher organisms substantially increases the effort to clone all the genes encoding the complete biosynthetic machinery for a given diterpenoid natural product. By contrast, genes encoding secondary metabolite biosynthesis in actinomycetes are nearly always arranged on the bacterial chromosome as a cluster. Recent characterization of terpene synthases (TSs) from several actinomycete species demonstrated that these enzymes are not membrane-bound and can be overproduced with relative ease as soluble, functional recombinant proteins in heterologous hosts such as *Escherichia coli* [9]. Diterpenoid biosynthesis in bacteria therefore may provide new opportunities to characterize these enzymes and to engineer their biosynthetic machinery for diterpenoid natural product structural diversity.

Diterpenoids are all derived from (*E*,*E*,*E*)-geranylgeranyl diphosphate (GGDP). Diterpene synthases (DTSs), also known as diterpene cyclases, catalyze the critical step in diterpenoid biosynthesis by morphing GGDP into one of the many diterpenoid scaffolds, further transformations of which by the downstream enzymes afford the enormous structural diversity known for diterpenoid natural products. TSs in general, DTSs included, can display incredible fidelity, catalyzing multi-step cyclization reactions with exquisite regiochemical and stereochemical control [10] or display marked product promiscuity, with a single enzyme generating over 50 unique products from a single substrate [11•]. It is the fidelity and promiscuity in this chemistry that has inspired a great interest in exploiting TSs for engineered biosynthesis of novel terpenoid natural products [7,8,12••].

Here we will first survey diterpenoid natural products of bacterial origin and briefly review their biosynthesis with emphasis on DTSs that channel GGDP to various diterpenoid scaffolds. We will then highlight differences of DTSs of bacterial and higher organism origins and discuss the challenges in discovering novel bacterial DTSs. We will conclude by discussing new opportunities for DTS mechanistic enzymology and applications of bacterial DTS in biocatalysis and metabolic pathway engineering.

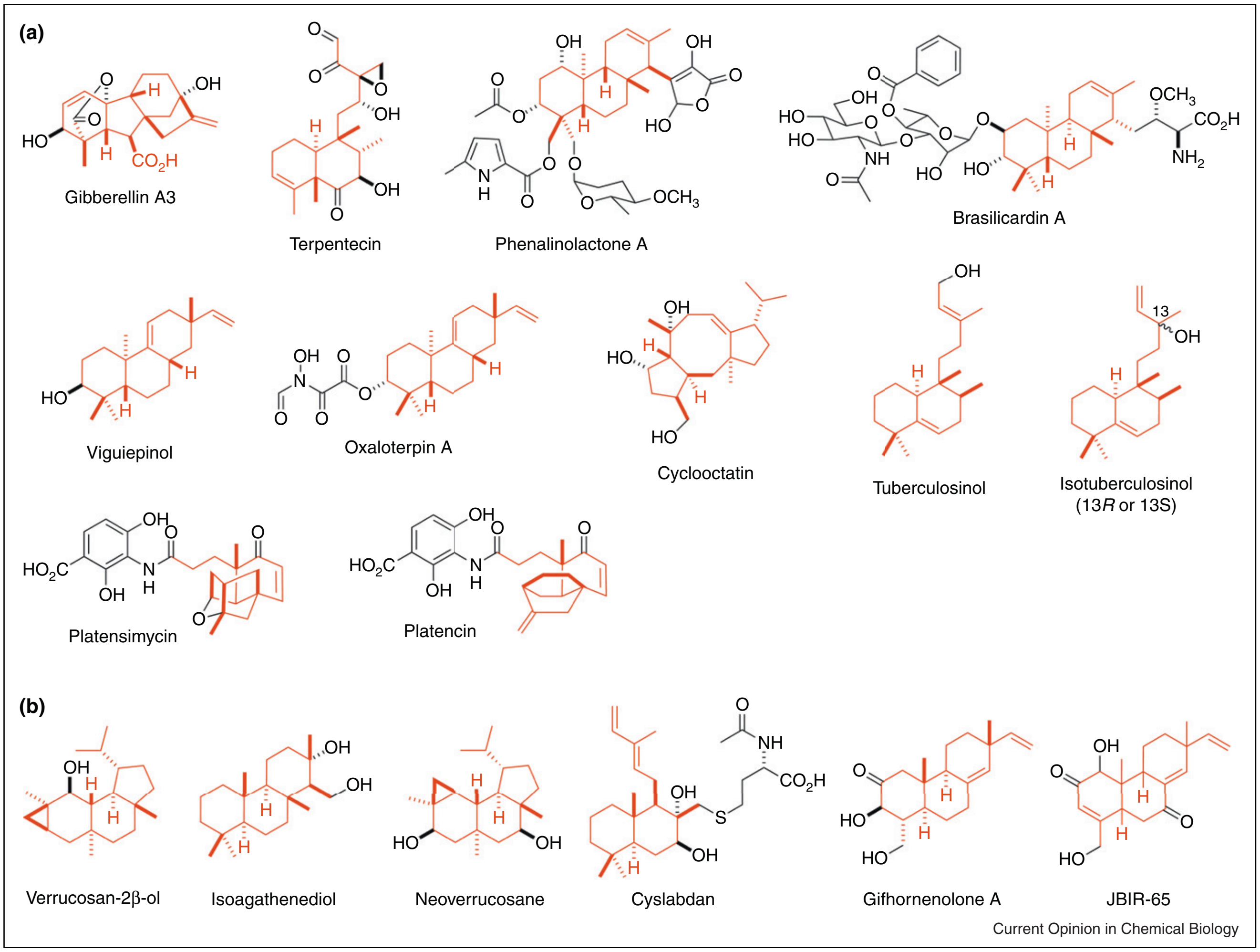
Bacterial diterpenoids

The discovery of gibberellins (GAs) from *Rhizobium phaseoli* in 1988, originally only known to fungi and plants, may represent the first report of bacterial diterpenoids [13,14]. It was followed by the discovery of verrucosan-2β-ol from *Chloroflexus aurantiacus* in 1993 [15] and iso-agathenediol from *Rhodospirillum rubrum* in 1995 [16]. Since then, the list of bacterial diterpenoids has grown steadily, and Figure 1 summarizes the bacterial diterpenoids known to date. These include terpentecin from *Streptomyces griseolosporeus* MF730-N6 [17,18••,19], the phenalinolactones from *Streptomyces* sp. Tu6071 [20], the brasilicardins from *Nocardia brasiliensis* IFM 0406 [21], viguiepinol and the oxaloterpins from *Streptomyces* sp. KO-3988 [22,23], cyclooctatin from *Streptomyces melanosporofaciens* MI614-43F2 [24], tuberculosinol and the isotuberculosinols from *Mycobacterium tuberculosis* H37Rv [25,26•,27–29], platensimycin from *Streptomyces platensis* MA7327 [30••,31,32], platencin from *Streptomyces platensis* MA7339 [33–35], the neoverrucosanes from *Saprospira grandis* [36,37], cyslabdan from *Streptomyces* sp. K04-0144 [38], the gifhornenolones from *Verrucosispora gifhornesis* YM28-088 [39], and JBIR-65 from *Actinomadura* sp. SpB081030SC-15 [40]. The actinomycetes have emerged as prolific producers of bacterial diterpenoids [1,2]. Bacterial producers of paclitaxel have also been reported, many of which were actinomycetes, however definitive evidence supporting their paclitaxel production remains elusive [41].

Bacterial DTSs

DTS classification follows other TSs. Type I TSs initiate a cyclization reaction via a heterolytic cleavage of the polyprenyl diphosphate, while type II TSs initiate the cyclization reaction via protonation of a double bond or an

Figure 1



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Bacterial diterpenoid natural products with their diterpenoid carbon scaffolds highlighted in red: **(a)** the biosynthetic gene clusters for these natural products have been cloned and partially characterized and **(b)** biosynthesis for these natural products has not been studied.