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REVIEW

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# Non-peptide Metabolites from the Genus *Bacillus*

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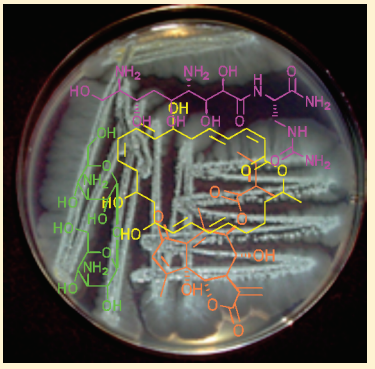
**ABSTRACT:** *Bacillus* species produce a number of non-peptide metabolites that display a broad spectrum of activity and structurally diverse bioactive chemical structures. Biosynthetic, biological, and structural studies of these metabolites isolated from *Bacillus* species are reviewed. This contribution also includes a detailed study of the activity of the metabolites described, especially their role in biological control mechanisms.



## INTRODUCTION

Studies of the natural products chemistry of microorganisms have indicated that bacteria and fungi are prolific sources of structurally unique, highly bioactive, and biomedically useful secondary metabolites. Many of these compounds have proven to be effective as chemotherapeutic agents in the treatment of human and animal diseases.[1] The fact that there is a continuing international focus on microbial products by pharmaceutical and agrochemical industries reflects their importance in the development of new therapeutic agents.[2,3]

It is also known that fungal pathogens cause devastating losses of crops and postharvest fruits throughout the world. Synthetic fungicides have long been employed to control these pathogens. However, their efficiency is decreasing owing to the development of resistant pathogens, which has intensified the search for safer methods to control these invasive organisms.[4] Among these alternatives, biocontrol is now an established subdiscipline in the science of plant pathology.[5] As biocontrol agents, some natural antagonistic microorganisms have shown to be effective against various pathogens.[6]

*Bacillus* species are well known for their ability to control plant diseases through various mechanisms, including the production of secondary metabolites.[7,8] Since *Bacillus* species are characteristically omnipresent in soils, they exhibit high thermal tolerance and rapid growth in liquid culture, and they readily form resistant spores and are considered to be a safe biological agent, their potential as biocontrol agents is considered high.[9]

Discovery of new natural products of relevance to humankind demands that freshly isolated organisms should be preserved for future use, as many microorganisms are isolated once or only rarely.[10] Thus, culture collections have the crucial role of providing the authenticated biological material upon which high-quality research is based.[11]

The World Federation for Culture Collections (WFCC) is concerned with the collection, authentication, maintenance, and distribution of cultures of microorganisms and cultured cells. A congress is held every four years to discuss advances in technology and common policies with regard to biodiversity and the role of culture collections.[11] The WFCC has a total membership of around 700 from 68 countries. One of the most prestigious culture collections is the American Type Culture Collection (ATCC), which has the most diversified assemblage of prokaryotes in the world, containing more than 18 000 strains in more than 750 genera. As a biological resource center, ATCC authenticates microorganisms and cell lines and manages logistics of long-term preservation and distribution of cultures for the scientific community. ATCC supports the cultures it acquires and authenticates with expert technical support, intellectual property management, and characterization data (http://www.atcc.org). Many strains described in this review have been classified by ATCC by means of a number, which indicates their origin and identification, such as *B. subtilis* 6051, *B. subtilis* 23059, and *B. subtilis* 23856.[12]

While most of the secondary metabolites of the genus *Bacillus* are peptide antibiotics, several species produce other compounds that are proving to be effective agents against molds and yeasts. Biological, biosynthetic, and structural studies of these non-peptide metabolites isolated from *Bacillus* species are reviewed herein.

## POLYKETIDES

Polyketides are a large family of secondary metabolites that form the basis of numerous human and veterinary drugs. The

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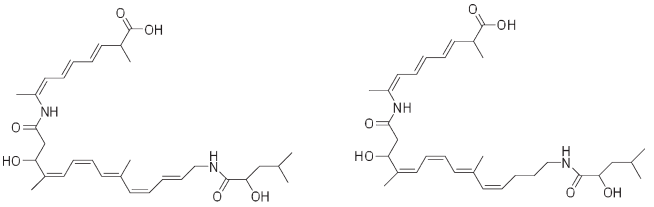
biosynthesis of complex polyketides is carried out by polyketide synthases (PKSs), enzymes composed of multifunctional polypeptides that are assembled into large protein complexes.[13]

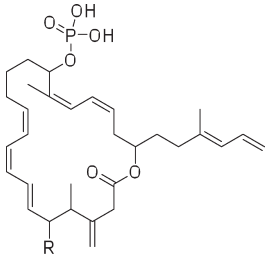
Bacillaene (**1**), a polyene antibiotic, was discovered as a component of the fermentation broth of several wild-type strains belonging to *B. subtilis*[14] and *B. amyloliquefaciens*.[15] It is a linear molecule with two amide bonds: the first links a α-hydroxy carboxylic acid to a ω-amino carboxylic acid containing a conjugated hexaene, and the second links the hexaene-containing carboxylic acid to an (ω-1) amino carboxylic acid containing a conjugated triene.[16] Bacillaene (**1**) inhibits prokaryotic but not eukaryotic protein synthesis by means of an unknown mechanism, and it exhibits high bacteriostatic activity against a wide spectrum of bacteria. Thus, this compound displays antimicrobial activity against human pathogens such as *Serratia marcescens*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*.[14] Recently, it was demonstrated that the hybrid polyketide/non-ribosomal peptide synthase is involved in the synthesis of a series of extremely labile, open-chained isomers with bacillaene (**1**) and dihydrobacillaene (**2**) as the most abundant representatives.[16] The final steps of bacillaene (**1**) biosynthesis in *B. amyloliquefaciens* FZB42 were recently elucidated by Piel et al.,[17] providing direct evidence for β,γ dehydration by *trans*-acyltransferase polyketide synthase.



**1** **2**

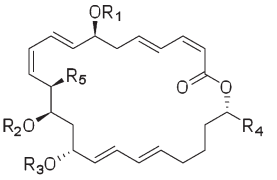
Difficidin (**3**) and oxidifficidin (**4**), detected in the culture broths of *B. subtilis*[18] (ATCC 39320 and ATCC39374) and *B. amyloliquefaciens* (FZB42),[19] are highly unsaturated 22-membered macrocyclic polyene lactone phosphate esters with broad-spectrum antibacterial activity.[20] Difficidin (**3**) was recently shown to be promising in its suppressive action against the enterobacterium *Erwinia amylovara*, a devasting plant pathogen causing necrotrophic fire blight disease affecting apple, pear, and other rosaceous plants.[19]

The primary mode of action of difficidin (**3**) is the inhibition of protein synthesis. It has proved to be highly bactericidal to both growing and stationary phase cultures and was found to inhibit protein synthesis more rapidly than RNA, DNA, or cell-wall synthesis in growing cells.[21]



**3** R = H
**4** R = OH

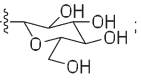
In addition to bacillaene (**1**) and difficidin/oxidifficidin (**3**/**4**), a third polyketide with a macrolide-like structure, macrolactin, originally detected in an unclassified deep-sea marine bacterium, has previously been reported from several other *Bacillus* strains.[22] The macrolactin carbon skeleton contains three separate diene structure elements in a 24-membered lactone ring.[1] Until now, at least 17 macrolactins have been described, and one of them, 7-*O*-malonylmacrolactin A (**6**), has recently been reported as being effective against Gram-positive bacterial pathogens.[23] Four members of the macrolactin family were detected in *B. amyloliquefaciens* FZB42: macrolactin A (**5**), macrolactin D (**10**), and 7-*O*-malonyl- (**6**) and 7-*O*-succinylmacrolactin A (**7**).[16] Macrolactin N (**13**) is a 24-membered lactone isolated by Yoo et al.[24] from *B. subtilis*, which showed antibacterial activity against *Echerichia coli*, *B. subtilis*, and *Staphylococcus aureus*. This lactone may serve as the prototype member of a new class of peptide deformylase inhibitors for the development of antibacterials because it significantly inhibited the *Staphylococcus aureus* peptide deformylase.

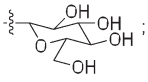


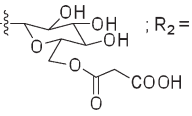
**5** $R_1 = R_2 = R_3 = R_5 = H$; $R_4 = CH_3$

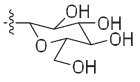
**6** $R_1 = COCH_2COOH$; $R_2 = R_3 = R_5 = H$; $R_4 = CH_3$

**7** $R_1 = COCH_2CH_2COOH$; $R_2 = R_3 = R_5 = H$; $R_4 = CH_3$

**8** $R_1$ =  ; $R_2 = R_3 = R_5 = H$; $R_4 = CH_3$

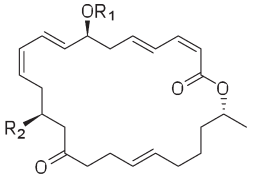
**9** $R_1 = R_2 = R_5 = H$; $R_3$ =  ; $R_4 = CH_3$

**10** $R_1$ =  ; $R_2 = R_3 = R_5 = H$; $R_4 = CH_3$

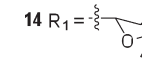
**11** $R_1$ =  ; $R_2 = R_3 = R_5 = H$; $R_4 = CH_2CH_3$

**12** $R_1 = R_2 = R_3 = H$; $R_4 = CH_3$; $R_5 = OH$

In the process of screening for peptide deformylase *S. aureus* inhibitors, four glycosylated macrolactin compounds, macrolactins O (**14**), P (**11**), Q (**15**), and R (**16**), along with the known macrolactins B (**8**) and C (**9**), were isolated from liquid cultures of *Bacillus* sp. AH159-1. They inhibited *S. aureus* peptide deformylase and also exhibited antibacterial activity against *E. coli* and *S. aureus*.[25]



**13** $R_1 = R_2 = H$

**14** $R_1$ =  ; $R_2 = OH$

In 2008, a new 24-membered macrolide, macrolactin T (**17**), and a new polyene δ-lactone, macrolactin U (**18**), along with macrolactins A (**5**), B (**8**), D (**10**), S (**12**), and O (**14**), were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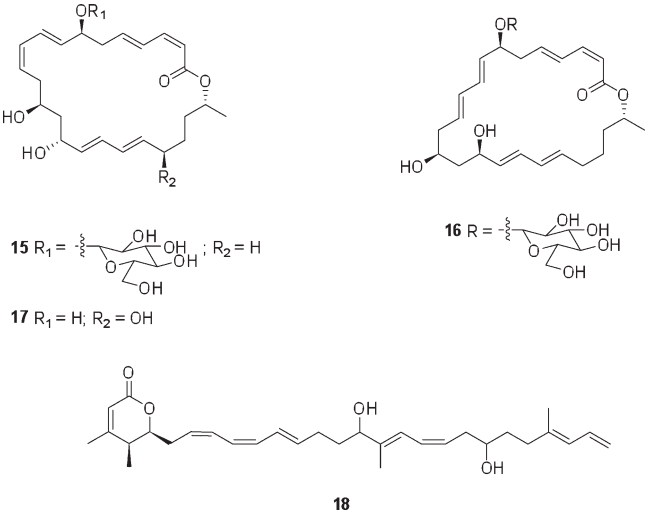
isolated from the cultured broth of *B. marinus*, which was isolated from *Suaeda salsa* collected along the coast of the Bohai Sea, adjacent to the People's Republic of China. The inhibitory activity of macrolactins T (**17**), B (**8**), and D (**10**) against *Pyricularia oryzae* and *Alternaria solani* was also reported.[28]



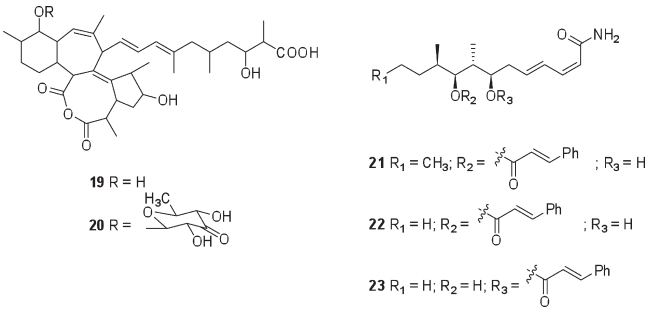

Several macrolactins were found to possess interesting pharmacological properties.[1] Macrolactin A (**5**), for example, was found to possess significant antiviral and cancer cell cytotoxic properties. Lactone **5** inhibited B16-F10 murine melanoma cell replication with in vitro $IC_{50}$ values of 3.5 $\mu$g/mL. Further, lactone **5** was a potent inhibitor of Herpes simplex type I virus (strain LL), as well as type II virus (strain G), with $IC_{50}$ values of 5.0 and 8.3 $\mu$g/mL, respectively.[1] Macrolactin A (**5**) was also tested by the National Cancer Institute for its potential utility in controlling human HIV replication (using human T-lymphoblast cells). Antiviral effects were recorded with maximum protection observed at concentrations of 10 $\mu$g/mL.

Aurantinins A (**19**) and B (**20**) are polyketide antibiotic complexes produced by *B. aurantinus* that exhibit antimicrobial activity against Gram-positive bacteria, especially anaerobes. Antibiotics **19** and **20** seem to be built up biosynthetically from two polyketide chains. A long chain originates from 11 acetate units, with three $C_1$ units arising from methionine, two $C_1$ units at C-5 and C-7 from acetate via decarboxylation, and one $C_1$ unit at C-1 from acetate being a starter unit. A short chain consisting of four acetate units in which a succinate is formed by "tail-to-tail condensation" of two acetate units might be the starter unit, with two $C_1$ units from methionine.[27]

Proticin is a phosphorus-containing, strongly unsaturated, amorphous compound that is produced by fermentation of a strain of *B. licheniformis*. It is especially active, in vitro, against a number of Gram-negative pathogens. The name proticin has been chosen to suggest a specific activity against *Proteus* species.[28,29]

Recently, the antibiotic YM-47522 (**21**) was isolated from the culture broth of *Bacillus* sp. YL-03709B and exhibited potent in vitro antifungal activity especially against *Rhodotorula acuta* and *Pichia angusta*. It also showed moderate or weak antifungal activity against *Candida albicans* and *Cryptococcus neoformans*.[30,31]

Basiliskamide A (**22**) and basiliskamide B (**23**), antifungal metabolites that show potent activity against *C. albicans*, are produced by *B. laterosporus*.[32] Basiliskamide A (**22**) also showed at least 4-fold less cytotoxicity to normal human fibroblast cells than amphotericin B. The linear polyketide chain in basiliskamide A (**22**) is one methylene unit shorter than the corresponding polyketide chain in the *Bacillus* metabolite YM-47522 (**21**). Biogenetically, this can be rationalized by the incorporation of a propionate starter unit in YM-47522 (**21**) and an acetate starter unit in basiliskamide A (**22**). Interestingly, the loss of one carbon from the polyketide chain in **22** appears to result in a significant increase in activity against both *C. albicans* and *A. fumigatus*.




## PLANT GROWTH HORMONES

One group of secondary metabolites very important in biotechnology are the plant growth hormones, which are widely used in agriculture. Plant growth hormones, biosynthesized in minute amounts, affect many plant growth and development activities.[33,34] Not only are these hormones biosynthesized by higher plants, they are also produced by lichens,[35] mosses,[36] fungi,[37] and bacteria.[38]

Thus, *Bacillus* spp. are thought to enhance plant growth through the biosynthesis of different plant growth hormones.[39] It has been reported that *B. megaterium* and *B. cereus* synthesize as secondary metabolites plant growth regulators as the auxin, gibberellin, cytokinin, and abscisic acid types when in peptone-rich growth medium.[40] Also representatives of the *B. subtilis/B. amyloliquefaciens* group produce substances with auxin indole-3-acetic acid (IAA)-like bioactivity,[41] and *B. amyloliquefaciens* FZB42 produces reasonable amounts of IAA when fed tryptophan.[42]

It has also been reported that *B. edaphicus* (CGMCC No. 2315) can improve soil quality, secrete plant growth hormones (indolepropionic acid, zeatin, and gibberellin), and prevent and treat fungal diseases and nematode infestations.[43] The strain, with high resistance to salt, heat, and stress, can be used to prepare biological organic fertilizer, ecological fertilizer, a biological pesticide, and soil oil amendment substance.[43]

Moreover, it has been shown that a blend of volatile compounds, especially 3-hydroxybutan-2-one (acetoin) and butane-2,3-diol, released by the rhizobacteria *B. subtilis* and *B. amyloliquefaciens* can enhance plant growth.[44]

## TERPENOIDS

Isoprene is the smallest representative of the natural product group of terpenoids. In contrast to other bacteria, *B. subtilis* emits the volatile compound isoprene in relatively high concentrations, as has been described for *B. subtilis* 6051, *B. subtilis* 23059, and *B. subtilis* 23856.[12]

Isoprenoid metabolites are produced also by the acidophilic, thermophilic *B. acidocaldarius*. In addition to normal bacterial isoprenoids such as menaquinone and polyprenols, squalene and pentacyclic hopane triterpenes were also isolated and characterized.[45]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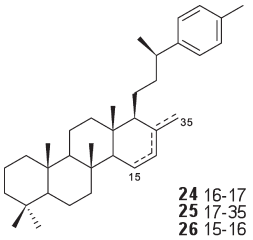
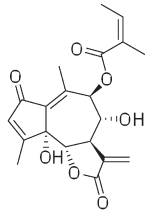
Sporulenes A (**24**), B (**25**), and C (**26**) are three pentacyclic terpenoids, isolated from *B. subtilis* spores,[46] which may protect *Bacillus* spores against oxidative stress. They are produced by cyclization of regular polyprenes, a reaction that is more favorable chemically than the formation of terpenoids such as hopanoids and steroids from squalene. The biological role(s) of the sporulenes is related to sporulation since *B. subtilis* produces them only when producing spores. There is a clear association between sporulene production and the relief of oxidative stress, since wild-type spores of *B. subtilis* resist oxidative stress more successfully than spores of a mutant strain that cannot produce sporulenes. Sporulenes may protect *B. subtilis* spores against oxidative stress in two ways: one physical and the other chemical. Physical protection could come from a protective barrier made by packing sporulenes into cellular membranes. The reduced sporulenes could alternatively provide a chemical barrier as oxygen scavengers through the oxidative conversion of their cyclohexadiene unit to a phenyl moiety.

Pumilin (**27**) is a sesquiterpene lactone antibiotic isolated from submerged cultures of *B. pumilus*. It is active against Gram-positive organisms only,[47] but it also shows high cytotoxic activity in bioassays against three human cancer cell lines (brain, mammary gland, and lung).[48]




**24** 16-17
**25** 17-35
**26** 15-16



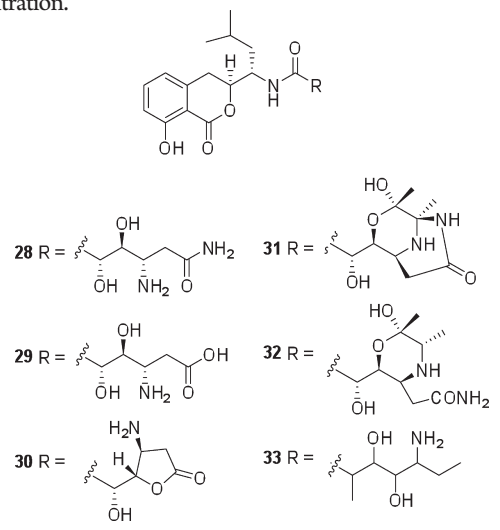

**27**

## ISOCOUMARINS

The isocoumarins represent an interesting group of phenolic compounds and occur in *Bacillus* species as phenylpropanol derivative substances having important biological activities. Eleven strains of *B. subtilis*, from a number of *Bacillus* strains isolated from different geographical and ecological niches, were found to produce amicoumacins A (**28**), B (**29**), and C (**30**), recognized as antibiotics of the isocoumarin group.[49] Amicoumacins, isolated for the first time from *B. pumilus*, exhibited antibacterial activity and suppressed inflammation and ulcer activity. Amicoumacin A (**28**) was mainly active against Gram-positive bacteria *Salmonella* sp. and *Shigella* sp. The antimicrobial activities of amicoumacins B (**29**) and C (**30**) are weak compared to that of amicoumacin A (**28**).[50]

Two 8′-phospho derivatives of amicoumacins A and B were isolated from the culture broth of a strain of *B. pumilus* together with amicoumacins A (**28**) and B (**29**). Their structures were elucidated on the basis of spectroscopic methods and treatment with alkaline phosphatase. Comparison of the antibacterial activities of these compounds against methicillin-resistant *Staphylococcus aureus* (MRSA) suggests that the C-8′ hydroxy and C-12′ amide groups of amicoumacin A (**28**) play a critical role in anti-MRSA activity.[51]

Two novel isocoumarins related to amicoumacin, bacilosarcins A (**31**) and B (**32**), were isolated from a culture broth of the marine-derived bacterium *B. subtilis* TP-B0611. Bacilosarcin A (**31**) possesses an unprecedented 3-oxa-6,9-diazabicyclo[3.3.1]nonane ring system, while bacilosarcin B (**32**) has a 2-hydroxymorpholine moiety that is rare in Nature. These compounds showed plant growth inhibition against barnyard millet. Bacilosarcin A (**31**) showed 82% inhibition at 50 mM against growth of barnyard millet sprouts, while bacilosarcin B (**32**) showed very weak activity at the same concentration.[52]




During a search for natural compounds active against viral replication, baciphelacin (**33**), an isocoumarin antibiotic produced by *B. thiaminolyticus*,[53] was found to be a potent inhibitor of protein synthesis in mammalian cells.[54]

## MISCELLANEOUS METABOLITES

Zwittermicin A (**34**) is a linear hybrid polyketide−nonribosomal peptide that has an unusual chemical structure inclusive of a D-amino acid and ethanolamine and glycolyl moieties, as well as having an unusual terminal amide generated from the modification of the non-proteinogenic amino acid $\beta$-ureidoalanine. Bioinformatics and biochemical analyses of the zwittermicin A (**34**) biosynthetic enzymes have suggested that zwittermicin A (**34**) is biosynthesized initially as part of a larger metabolite processed twice, resulting in the formation of zwittermicin A (**34**) and two additional metabolites.[55] This compound was first identified for its role in the suppression of plant disease by *B. cereus* UW85.[56,57] It has a broad spectrum of activities, inhibiting certain Gram-positive, Gram-negative, and eukaryotic microorganisms.[58] Zwittermicin A (**34**) also enhances the insecticidal activity of the protein toxin produced by *B. thuringiensis*,[59] increasing the mortality rate of particularly robust insects such as gypsy moths reared on willow leaves.[60]

An aminosugar antibiotic, 3,3′-neotrehalosadiamine (3,3′-diamino-3,3′-dideoxy-$\alpha$,$\beta$-trehalose) (**35**), has been found to be produced by a bacterial culture identified as *B. pumilus*. This compound inhibited Gram-positive and Gram-negative bacteria and proved to be nontoxic to mice at 400 mg/kg in vitro.[61,62] Neotrehalosadiamine (**35**) was also isolated from a *B. circulans* fermentation broth[63] and shown to function as an autoinducer for glucose uptake regulation in *B. subtilis*.[64] Recent reports have described the purification, crystallization, and preliminary X-ray analysis of a putative pyridoxal phosphate-dependent aminotransferase from *B. subtilis* required for the biosynthesis of 3,3′-neotrehalosadiamine (**35**).[65]

The antibiotic kanosamine (**36**), produced by *B. cereus* UW85, was identified as 3-amino-3-deoxy-D-glucose. It proved to be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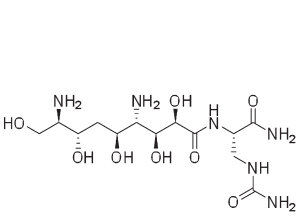
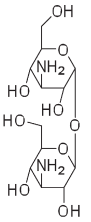
highly inhibitory to the growth of plant-pathogenic oomycetes and moderately inhibitory to certain fungi and several bacterial species tested.[66] The antibiotic kanosamine (**36**) inhibited the growth of *Saccharomyces cerevisiae* and a range of human pathogenic fungi including *Candida albicans*. The action of kanosamine (**36**) on *C. albicans* cells resulted in profound morphological changes, inhibition of septum formation, and cell agglutination.[67]



**34**



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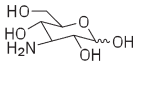


**36**

*B. sphaericus*, a spore-forming, chemoheterotrophic, and aerobic bacterium, has been shown to produce a mosquito-larvicidal binary toxin with activity against several species of mosquito, including species that transmit malaria and filarial disease.[68] *B. sphaericus* binary toxin is a mosquito-larvicidal crystal protein produced during the sporulation phase of growth. The toxin is composed of two proteins, BinA and BinB, that require each other for full activity, and maximum activity is obtained when both components are present in equimolar ratio.[69] After ingestion by susceptible larvae, the binary toxin crystal is solubilized and processed by proteases in the larval gut, after which BinB binds to a specific receptor on the epithelium cell membrane. BinA then binds to BinB or the BinB receptor complex and translocates into the cytosol, exhibiting its activity via an unknown mechanism.[70]

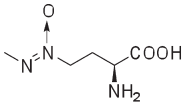
Active strains of *B. thuringiensis* subsp. *israelensis*[71] are also known to produce four insecticidal crystal proteins that are solubilized under alkaline conditions of the insect midgut and proteolytically activated from a protoxin into a toxin with specific binding properties to different receptor molecules on the midgut epithelium of mosquitoes.[72]

*B. cereus* is becoming one of the most important causes of food poisoning in the industrialized world.[73] This bacterium causes two types of gastrointestinal disease, diarrheal and emetic syndromes caused by different types of toxins. The emetic toxin, causing vomiting, has been characterized as a small ring-formed peptide (cereulide),[74] while the diarrheal disease is caused by one or more protein enterotoxins thought to elicit diarrhea by disrupting the integrity of the plasma membrane of epithelial cells in the small intestine. The three toxins that have been identified as etiological agents of the diarrheal disease are the pore-forming cytotoxins hemolysin BL, nonhemolytic enterotoxin, and cytotoxin K. These cytotoxins are part of a virulence regulon that is activated by the transcriptional regulator PlcR; however, it is becoming increasingly evident that other regulatory factors are involved, playing a role in determining the pathogenic potential of individual strains.[75,76]

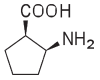
An antifungal protein, designated as bacisubin, was isolated from a culture of *B. subtilis* strain B-916. The protein exhibited inhibitory activity on mycelial growth in *Magnaporthe grisease*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Alternaria oleracea*, *A. brassicae*, and *Botrytis cinerea*. Bacisubin demonstrated neither protease activity nor protease inhibitory activity but did manifest ribonuclease and hemagglutinating activities.[77]

An azoxy-containing antifungal agent named azoxybacilin (**37**) was found in the culture broth of *B. cereus* Frankland and Frankland NR2991. Azoxybacilin (**37**) shows potent antifungal activity in vitro in an amino acid-free medium, especially against mycelial fungi such as *Aspergillus fumigatus* and *Trichophyton mentagrophytes*.[78] This antibiotic is different in its mechanism of action from other antifungal compounds used in hospital clinics or that have been reported in the literature, because it exhibits such activity by interfering with the regulation of the expression of sulfite reductase activity. Azoxybacilin (**37**) could be the first example of an antifungal agent that acts as a gene regulation inhibitor and provides a new way of inhibiting fungal cells.[79]

Cispentacin was isolated from the culture broth of *B. cereus* strain L450-B2.[80] Its structure was determined by spectroscopic analysis and chemical synthesis as (1*R*,2*S*)-2-aminocyclopentane-1-carboxylic acid (**38**). This compound exhibited weak inhibitory activity against *Candida albicans* A9540 and *Cryptococcus neoformans* IAM 4514 only in certain media and no activity against other fungi and bacteria.[81] Biocerin, another antibiotic produced by *B. cereus*, inhibited Gram-positive and Gram-negative bacteria at concentrations of 0.5 and 1.0 mg/mL. All bacterial species tested were inhibited by a concentration of 1.0 mg/mL. *Salmonella paratyphi* A, *Salmonella paratyphi* B, and *Sarcina lutea* were the only species not inhibited by a concentration of 0.5 mg/mL. The lower concentrations of 0.1 and 0.05 mg/mL did not inhibit any of the species tested except *Staphylococcus aureus* and *Staphylococcus albus*.[82]



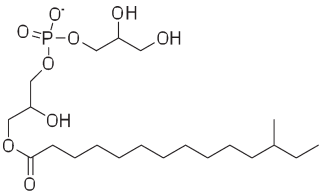
**37**



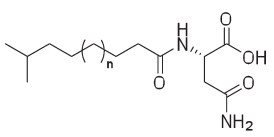
**38**

Bacilysocin (**39**), a phospholipid antibiotic that accumulates within the cells of *B. subtilis* 168, commenced its production immediately after growth ceased and before the formation of heat-resistant spores. The structure of **39** is 1-(12-methyltetradecanoyl)-3-phosphoglyceroglycerol, and this compound exhibits antimicrobial activity, especially against certain fungi. Its activity was more pronounced against the eukaryotic organism *Saccharomyces cerevisiae*, in addition to the fungi *Candida pseudotropicalis* and *Cryptococcus neoformans*, which are characterized by nonfilamentous growth.[83]

A screening program of marine bacteria for antimicrobial activity resulted in the isolation of *B. pumilus* (SP21) from a sediment sample collected in the Bahamas. Bioassay-guided fractionation led to the isolation of three antibiotic compounds, named lipoamides A−C (**40**−**42**). These compounds were screened for antimicrobial activity against *Staphylococcus aureus* (ATCC 10832), *Pseudomonas aeruginosa* (ATCC 14210), and *Candida albicans* (ATCC 14053). Lipoamides showed no significant antibacterial activity against *S. aureus* and *P. aeruginosa* with MIC values above 100 $\mu$g/mL.[84]



**39**



**40** n = 3
**41** n = 4
**42** n = 5

## CONCLUSIONS

*Bacillus* species are both taxonomically and metabolically diverse, and they exhibit enormous metabolic capabilities and versatile biochemistry through the production of structurally

diverse bioactive chemical structures. Although *Bacillus* species mainly synthesize peptides, antibiotics belonging to other chemical classes are also produced by these microorganisms, and the determination of their chemical structure and the mechanism of their biological action, which have been summarized in this review, are of fundamental and practical interest.

Nature has developed strategies for the assembly of structurally diverse natural products derived from complex biosynthetic pathways. There have been numerous reports about the discovery of novel natural products from sequenced microbes by genomics-guided approaches. Mining these pathways in *Bacillus* genomes has revealed a greater biosynthetic potential for novel natural products than anticipated. One important challenge that lies ahead is the development of general methods for activating the expression of silent cryptic biosynthetic gene clusters.[85] In addition to the discovery of novel structures, the identification of new biosynthetic loci in *Bacillus* spp. has provided studies in a new direction, exploring the biological significances of compounds, and in same cases has revealed new aspects of the biology of the producing strain.

Systematic natural product isolation efforts and the application of those new technologies, proteomic, genomic, and transcriptomic, connected to genome mining approaches, will undoubtedly yield many more interesting molecules. Chemical and combinatorial biological diversification of these scaffolds will in turn afford promising lead compounds for future drug discovery.[85]

On the other hand, exciting and intriguing biosynthetic chemistry remains to be discovered, and it may be predicted that future exploration of novel gene clusters in this heterogeneous genus will expand the already remarkable spectrum of secondary metabolites isolated from *Bacillus* species. A complete study of the genome and secondary metabolome of these strains, growing in diverse niches, will also reveal the role of these metabolites in the ecological adaptations of these organisms.

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