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Review

Bacillus Metabolites: Compounds, Identification and Anti-*Candida albicans* Mechanisms

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Abstract: *Candida albicans* seriously threatens human health, especially for immunosuppressed groups. The antifungal agents mainly include azoles, polyenes and echinocandins. However, the few types of existing antifungal drugs and their resistance make it necessary to develop new antifungal drugs. *Bacillus* and its metabolites has antifungal activity against pathogenic fungi. This review introduces the application of *Bacillus* metabolites in the control of *C. albicans* in recent years. Firstly, several compounds produced by *Bacillus* spp. are listed. Then the isolation and identification techniques of *Bacillus* metabolites in recent years are described, including high-precision separation technology and omics technology for the separation of similar components of *Bacillus* metabolites. The mechanisms of *Bacillus* metabolites against *C. albicans* are distinguished from the inhibition of pathogenic fungi and inhibition of the fungal virulence factors. The purpose of this review is to systematically summarize the recent studies on the inhibition of pathogenic fungi by *Bacillus* metabolites. The review is expected to become the reference for the control of pathogenic fungi such as *C. albicans* and the application of *Bacillus* metabolites in the future.

Keywords: anti-*Candida albicans*; *Bacillus* spp.; identification; metabolites; virulence factors

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1. Introduction

In recent years, the number of immunosuppressed patients has increased. The incidence of deep pathogenic fungi infection in these patients has increased dramatically, becoming one of the main causes of death. A variety of *Candida* spp., *Cryptococcus neoformans*, *Coccidium* spp., and *Aspergillus* spp. are the most common deep pathogenic fungi [1]. The prevalence rate of *Candida* spp. was 7.6% in the hospital blood samples, ranked fourth among all pathogens and first among pathogenic fungi. 95% of *Candida* infections are caused by *C. albicans*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*. The detection rate of *C. albicans* is the highest at more than 50% [2,3]. *C. albicans* is one of the conditional pathogenic fungi that can cause severe deep infection. It can cause skin, mucosal and systemic infections in patients with low immune function, or malignant tumors, and can lead to death in severe cases. *C. albicans* is eukaryotic, with which the development of antifungal drugs is limited [4]. In addition, traditional antifungal drugs have high selectivity, which might cause drug resistance. Therefore, it is urgent to propose new strategies for the treatment of *C. albicans* infection.

Many kinds of drug molecules can prevent the growth or reduce pathogenicity of *C. albicans;* however, the overuse of broad-spectrum antibiotics to control human pathogenic fungi could greatly accelerate the development of pathogenic fungal antibiotic resistance. Drug molecules are prone to metabolic inactivation in vivo, and some novel antifungal drugs (such as new azoles) also have toxic side effects [5]. Therefore, current antifungal strategy research has been focused on finding more drug sources. Natural products are one of the important sources of new drug discovery, as they can provide a variety of bioactive chemical entities to develop new drugs. Resources of natural products are abundant, and natural products are conducive to the discovery of new targets and new pathways. Some

drugs currently used clinically to treat fungal infections are also derived from natural products, e.g., polyene compounds and echinocandins [6,7]. In addition, some peptides, amino acids, macrolides, terpenes, alkaloids, saponins, sterols and heterocyclic compounds in natural products exhibit activity against the pathogenic fungi [8–10].

An important source of natural products is the microbial metabolites. The microorganisms with antifungal activity mainly include *Trichoderma* spp., *Saccharomyce* spp., *Bacillus* spp., *Pseudomons* spp. and *Streptomyces* spp. Among them, *Bacillus* spp. is widely used for antifungal action because of its good biosafety and strong resistance. *Bacillus* spp. is Gram-positive bacteria widely existing in various living environments; it can produce endophytic spores and has a fast reproduction speed, strong environmental adaptability, a resistance to stress and wide antibacterial spectrum. It is also a common endophyte in plants and is non-toxic to humans and animals. *Bacillus* spp. can produce a variety of bioactive substances with antifungal properties, including lipopeptides, enzymes, bacteriocins, polyketides and volatile compounds, which have important applications in the control of pathogenic fungi, especially *C. albicans*.

2. Inhibition of *Bacillus* Metabolites on *C. albicans*

2.1. Lipopeptides

Lipopeptide compounds produced by *Bacillus* spp. include surfactin, iturin and fengycin [11]. The chemical structures of these three lipopeptides are shown as Figure 1 [12]. The antifungal mechanism of surfactin is mainly to destroy the lipid membrane of fungi and lyse the pathogenic fungi [13]. Iturin and fengycin exhibit strong antifungal activity, of which the mechanism is to change the surface tension of fungal cell membrane; this results in the formation of micropores and the leakage of K^+ and other important ions in the cell, causing cell death [14,15]. Indonesian marine bacteria *Bacillus subtilis* C19 could produce surfactin, which could inhibit the growth of *C. albicans* [16]. Isolating lipopeptide C16-fengycin A from *Bacillus amyloliquefaciens* fmb60 could produce C16-fengycin A, which shows significant anti-*C. albicans* activity [17].

2.2. Enzymes

Cell wall lyases produced by *Bacillus* spp. exhibit antifungal activity against pathogenic fungi. The lyases include cellulase, glucanase, protease and chitinase. Because the major components of fungal cell wall are chitin and glucan, lyases produced by *Bacillus* spp. are particularly effective against fungi [18]. *Bacillus aryabhattai* isolated from the ocean can produce chitinases, which could convert chitin into chitin oligomers. The chitinases can be antifungal agents. The highest specific activity of the chitinases is 175.4 U/mL. The antifungal activity of chitinase against pathogenic fungi such as *C. albicans* and *Fusarium oxysporum* exhibits inhibition zone with diameter of 14 mm [19]. The unique chitinotrophic *Bacillus altitudinis* isolated on shrimp shell from salt lakes could produce a novel chitin-oligosaccharide material and thermostable chitinase (5.1 U/mL). Chitin-oligosaccharide and chitinase have synergistic antifungal activity against *Candida* spp. They can kill 50% of 10^6 cells in 6 h and were promising to be new antifungal agents without side effects [20]. The researchers revealed the molecular mechanisms of *Lactobacilli rhamnosus* GG inhibited mycelial morphogenesis, which was a key step in the virulence of *C. albicans*. The major peptidoglycan hydrolase Msp1 was identified as a key effector molecule. Msp1 exhibits antifungal activity due to its ability to degrade the major polymer chitin in the hyphal cell wall of *C. albicans* [21]. *Bacillus safensis* attaches to *C. albicans* physically and degrades rosary hyphae. The activity of bacterial chitinase to fungal cell wall chitin was proved by genetic and phenotypic analysis to be the factor leading to the antifungal activity of *B. safensis* [22].

2.3. Polyketones

Polyketone compounds produced by *Bacillus* spp. include bacillaene, difficidin and macrolactin. The chemical structures of these three polyketones are shown as Figure 2 [12]. Among these polyketones, macrolactin has antifungal activities against many pathogenic

fungi [23]. A new strain *B. amyloliquefaciens* isolated from a salty lake in Algeria can produce antifungal metabolites, including lipopeptides, polyketones and other new metabolites, which is proved by the gene clusters of the strain. The antifungal lipopeptides include surfactin and fengycin. The antibacterial polyketides include macrolactin and bacillaene, and the antifungal metabolite also include a putative novel lanthipeptide [24].

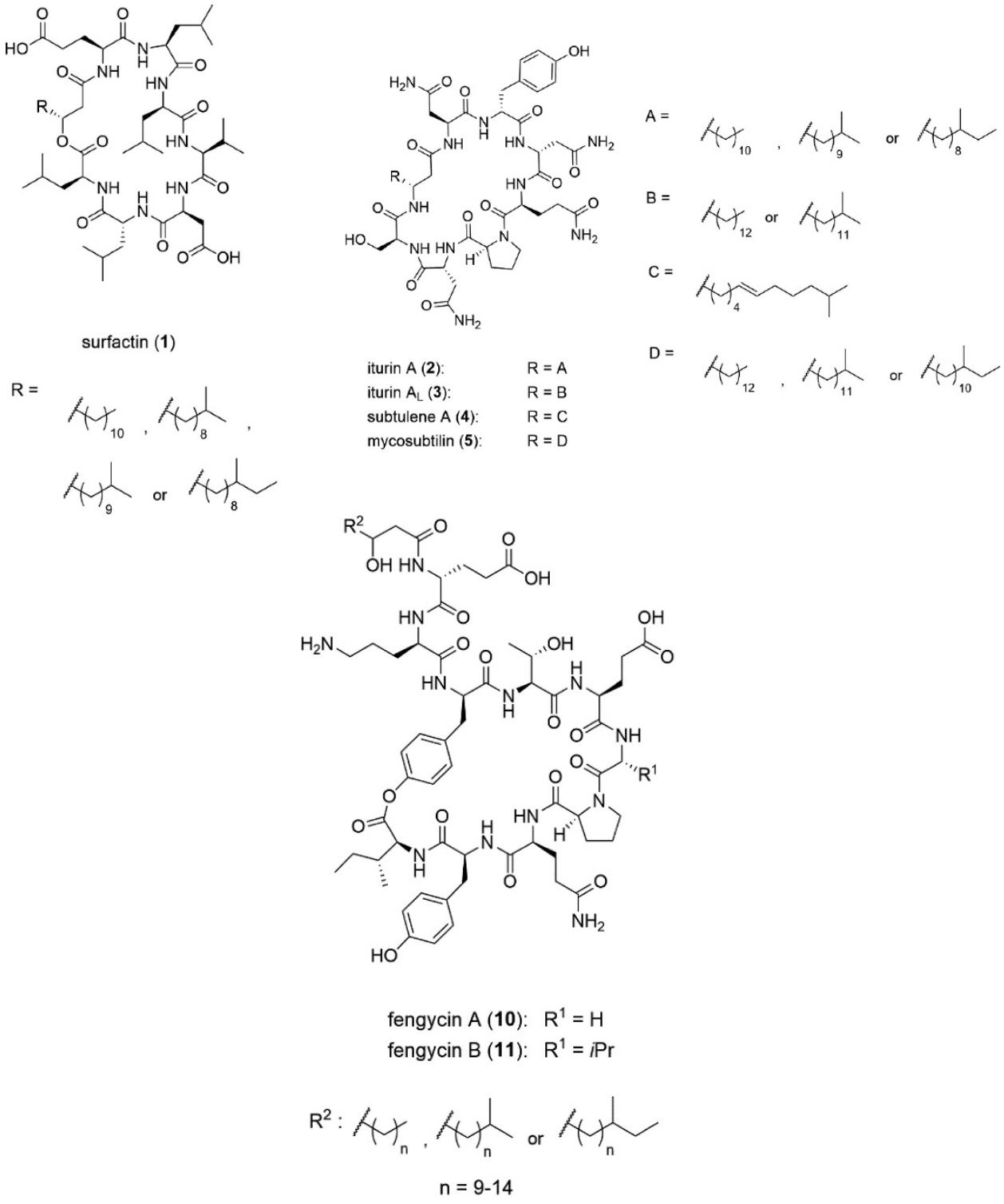

Figure 1. Chemical structures of surfactin, iturin and fengycin.

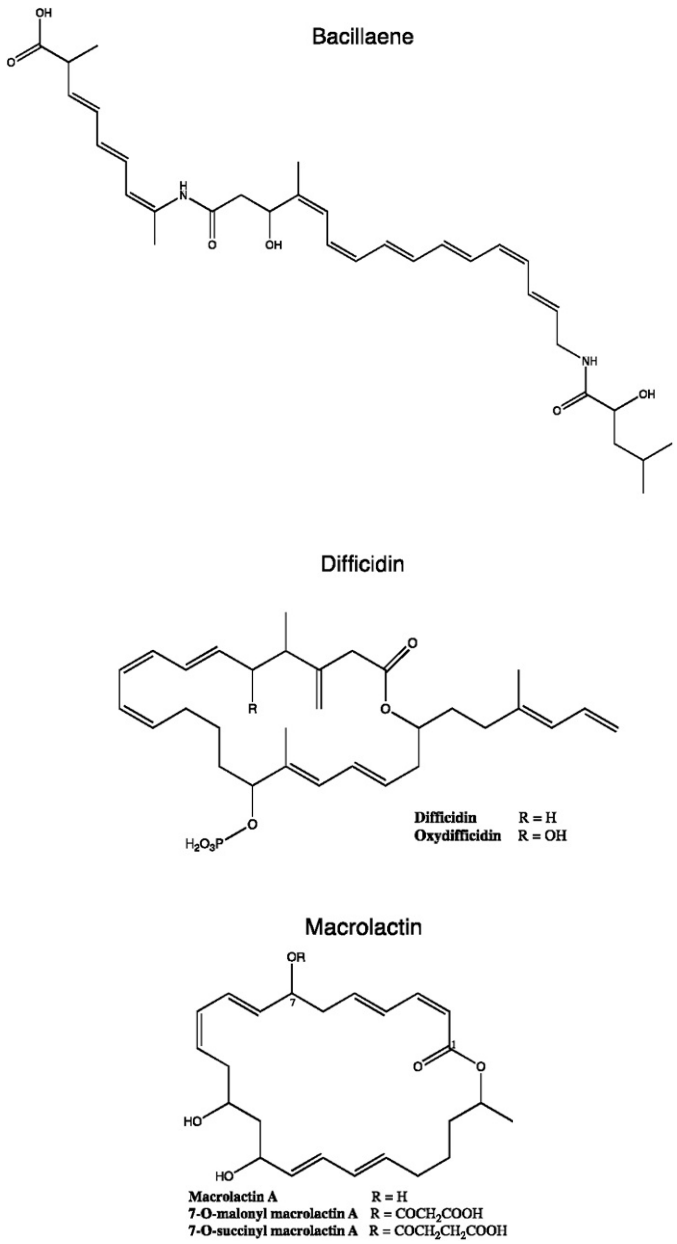

Figure 2. Chemical structures of bacillaene, difficidin and macrolactin.

2.4. Bacteriocins

Bacteriocin compounds produced by *Bacillus* spp. include lanthionine, subtilin and nisin A. The structures of these three bacteriocins are shown as Figure 3 [12]. Nisin A has antifungal effects by inhibiting cell wall synthesis and drilling holes on cell membranes. Its N-terminus binds to lipid II on the target cell membrane, and the N-terminus structure changes so that the C-terminus can be inserted into the target cell membrane, through which a pore is formed. The loss of small molecules such as K^+, inorganic phosphorus, glutamic acid and ATP from the pore causes bacterial death [25]. An anti-*C. albicans* bacteriocin produced by marine *Bacillus* sp. Sh10 was purified and characterized [26]. Some new kinds of bacteriocin produced by other bacteria also have anti-*C. albicans* activity. It was found that *Enterococcus faecalis* could inhibit *C. albicans*, which was mediated by EntV specificity. EntV is a kind of bacteriocin which can reduce the virulence and biofilm formation of *C. albicans* by inhibiting mycelial morphogenesis. The disulfide bonds formed in EntV are necessary for antifungal activity [27].

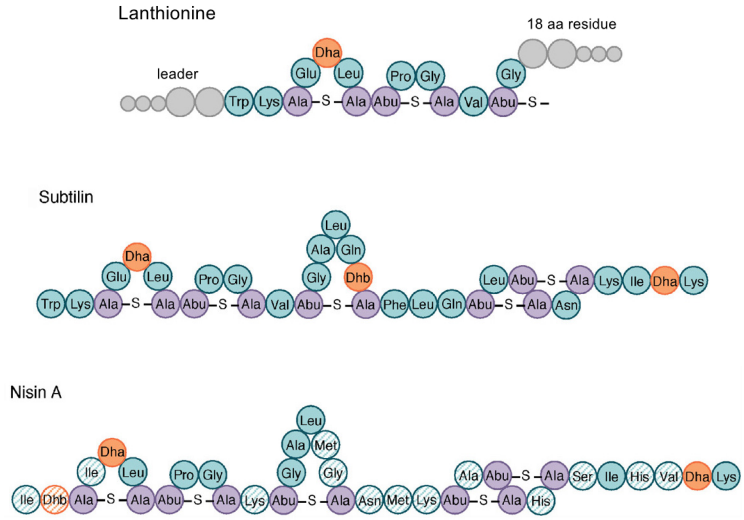

Figure 3. Structures of lanthionine, subtilin and nisin A.

2.5. Volatile Compounds

Volatile compounds include volatile inorganics (VICs) and volatile organic compounds (VOCs). VICs are the by-products of primary metabolism, which are the compounds containing carbon, hydrogen, sulfur or nitrogen, e.g., CO_2, CO, H_2, HCN, H_2S, N_2, NH_3 and NO. VOCs are small molecular compounds with carbon atoms less than 20, which have the characteristics of low molecular weight (100~500 Da), high vapor pressure, low boiling point and lipophilicity. Volatile pyrazine compounds produced by *B. subtilis* have antifungal and nematicidal activity [28]. VOCs produced by *B. amyloliquefaciens* strain S13 have antifungal activity, including anti-*C. albicans* [29].

In addition, *Bacillus* spp. can also produce other kinds of metabolites. If its metabolites are to be applied, the separation, purification and identification technology are crucial.

3. Purification and Identification of *Bacillus* Metabolites

3.1. Purification and Identification Technology of Bacillus Metabolites

The metabolites produced by *Bacillus* spp. have a strong ability to inhibit pathogenic fungi. Because of the similar structure and molecular weight of the metabolites, it is necessary to purify and identify the metabolites by high-precision separation strategy. There are many methods for the purification and structure confirmation of *Bacillus* metabolites, e.g., high-performance liquid chromatography (HPLC, Shimadzu, Kyoto, Japan), reverse-phase chromatography (RPC, Shimadzu, Kyoto, Japan), gel permeation chromatography (GPC, Shimadzu, Kyoto, Japan), mass spectrometry (MS, Bruker Daltonics, Bremen, Germany), nuclear magnetic resonance (NMR, Bruker, Madison, WI, USA) and infrared (IR) spectroscopy (Jasco Analytical, Tokyo, Japan) etc. The marine bacteria *Bacillus subterraneus* 11593 can produce a new indole alkaloid. By detailed analysis of its NMR spectroscopic data, and further by the theoretical ECD (electronic circular dichroism) calculations, the absolute configuration of the new compound was determined [30]. The metabolites of *Burkholderia* sp.by IR spectroscopy (Shimadzu, Japan) were analyzed. The stretching vibration of glycosidic bonds in the fingerprint area (1200 cm^{-1} to 800 cm^{-1}) of the IR spectroscopy confirmed that the biosurfactant produced by endophytic bacteria is glycolipid [31]. For some metabolites, simple extraction methods can also achieve better results. Marine bacterium *B. subtilis* has an antibacterial membrane effect against *C. albicans*. The metabolite was 5-hydroxymethyl-2-furaldehyde (5HM2F) by ethyl acetate extraction and mass spectrometry analysis [32]. A new bioactive compound from *Bacillus megaterium* was extracted, purified and identified. The bioactive compounds were extracted with *n*-butanol and purified on a TLC plate. Further structural confirmation indicated that it is a cyclic polypeptide with similar structure to bacitracin and broad-spectrum antibacterial

activity [33]. The anti-*C. albicans* bacteriocin produced by *Bacillus* Sh10 is purified by precipitation and gel chromatography. Through the purification process, the specific activity increased by 3.68 times, and the total activity recovery rate was 20.66%. The molecular weight of the compound was determined by SDS-PAGE, and the bacteriocin was analyzed by LC/MS/MS (Agilent Technologies, Santa Clara, USA) [26].

3.2. Combination of Purification and Identification Methods

The combination of purification and identification methods and the use of high-precision chromatography contributed to attain purified *Bacillus* metabolites. A new exopolysaccharide (EPS) produced by thermophilic *Bacillus haynesii* CamB6 was characterized by SEM-EDS (scanning electron microscopy-energy dispersive X-ray spectrometry, XMax-AZtec, Oxford Instruments), AFM (atomic force microscope, Bruker, Madison, WI, USA), HPLC (Shimadzu, Kyoto, Japan), GPC (Agilent Technologies, Santa Clara, CA, USA), FTIR (Fourier transform infrared spectroscopy, Jasco Analytical Spain, Madrid, Spain), NMR (Bruker, Madison, WI, USA) and TGA (thermogravimetric analysis, Cahn Scientific, Irvine, CA, USA). The analysis of GPC and HPLC indicated that EPS is a low molecular weight heteropolymer composed of mannose (66%), glucose (20%) and galactose (14%). FTIR analysis confirmed the properties of the polysaccharide, which were further confirmed by NMR spectroscopy [34]. By the analysis of HPLC-HRMS (HPLC-high resolution mass spectrometry, Bruker Daltonics, Bremen, Germany), the researchers found the lipopeptide produced by a new *Bacillus velezensis* strain DTU001. The antifungal activity of DTU001 is due to its ability to produce the lipopeptides that inhibit the proliferation of *C. albicans* [35].

3.3. Application of Omics and Other Methods in Purification and Identification of Bacillus Metabolites

In recent years, metabolomics has also been applied to the identification of microbial metabolites. According to the complex structure diversity of *Bacillus* metabolite, it is necessary to select appropriate metabolomics strategies, including chemical analysis techniques based on the nature of the target metabolite species. At present, the two most important types of microbial secondary metabolomics analysis platforms are based on NMR and MS analysis platform, which can perform efficient separation and identification of *Bacillus* metabolites [36]. Therefore, the combination of structural confirmation techniques such as NMR and MS with omics and theoretical calculations can more accurately attain the structural information of substances. Genome mining and metabolic analysis were used to identify the metabolites of *Bacillus siamensis* SCSIO 05476 from deep-sea sediments. The researchers first found candidate gene clusters that encode the biosynthesis of different secondary metabolites through genome mining, and further found a series of metabolites with strong antibacterial activity such as bacillibactins, fengycins, bacillomycins, surfactins, bacillaenes and macrolactins by LC-DAD-MS (Agilent Technologies, Santa Clara, CA, USA) [37]. The new *Bacillus* strain isolated from the salty lake was identified as *B. amyloliquefaciens* subsp. *plantarum* F11 by genomic sequence analysis, and showed that the strain carries a gene cluster for the production of many bioactive and surface-active compound. Activity-directed purification by hydrophobic interaction chromatography confirmed the ability of the strain to produce fengycin lipopeptides. Identification of the isolated fengycin homolog was by tandem mass spectrometry [24].

In addition, the computer technology also becomes a tool for auxiliary analysis and identification of the metabolites. The researchers developed an integrated computing program, MS-DIAL (Version 3.90), MS-FINDER (Version 1.62) and network-based tools, including GNPS (Global Natural Product Social Molecular Network) and MetaboAnalyst (MetaboAnalyst 3.0), for the analysis and identification of metabolites co-cultured with *Aspergillus sydowii* and *B. subtilis*. The accuracy of the new method was confirmed by purification and analysis of NMR data of seven new biosynthetic metabolites [38].

4. Mechanism of *Bacillus* Metabolites Inhibiting *C. albicans*

The possible mechanism of antifungal active substances inhibiting *C. albicans* is shown in Table 1, including inhibiting pathogenic fungi and inhibiting virulence factors. Systematic study of the mechanism can promote exploring more safe and effective antifungal drugs, laying a more substantial foundation for the selection of antifungal strategies.

Table 1. Possible mechanism of antifungal metabolites inhibiting *C. albicans*.

Inhibition of Pathogenic Fungi	Inhibition of Virulence Factors
(1) Damage of cell wall and cell membrane	(1) Inhibition of yeast to hypha biphasic transition
(2) Intracellular damage (Inhibition of target cell wall synthesis; Inhibition of cellular respiration and protein synthesis; Binding with fungal DNA)	(2) Inhibition of adhesion
	(3) Inhibition of pathogenic fungal invasion
	(4) Inhibition of hydrolase secretion
	(5) Inhibition of biofilm formation

4.1. Inhibition of Pathogenic Fungi

The inhibition mechanism of pathogenic fungi is divided into the damage of cell wall or cell membrane and intracellular damage.

4.1.1. Damage of Cell Wall and Cell Membrane

Bacillus metabolites can damage the cell wall or membrane structure of fungi, deform the cell wall structure, or affect the electrochemical potential of the membrane and the balance of ions, causing the leakage of cellular contents and eventually leading to fungal death. The cell wall is the first target for the binding of antifungal substances to fungi. The action and binding state of antimicrobial substances to cell wall are crucial [39,40]. The affinity of antifungal substances with cell wall or cell membrane is an important factor influencing its antifungal effect, which can be determined according to the different affinity so that appropriate antifungal drugs are selected [41,42]. Surfactin derived from *B. subtilis* has anti-*C. albicans* activity and reduces adhesion and morphogenesis. In addition, surfactin could also enhance fluconazole efficacy [43].

Various methods can show the damage of fungal cell wall and cell membrane. After AMP-17 treatment, the growth of *C. albicans* was significantly inhibited, which was observed by morphological methods (scanning electron microscope, etc.). The cells aggregated and dissolved, and the shape was seriously irregular. AMP-17 destroyed the integrity of *C. albicans* cell wall. Compared with untreated cells, the cell wall integrity of AMP-17 treated cells were only 21.7%. In addition, changes in membrane dynamics and permeability indicated that AMP-17 treatment destroyed the cell membrane. AMP-17 treatment can destroy the integrity of cell wall and the cell membrane structure of *C. albicans* [44]. The lipopeptide jagaricin can be effective against pathogenic fungi, by damaging the integrity of the membrane, which could cause rapid influx of Ca^{2+} or other ions into susceptible target cells. Jagaricin triggers cell wall enhancement, general closure of membrane potential-driven transport and upregulation of lipid transporters. The integrity of cell envelope is related to the effect of jagaricin [45]. The bacterial chitinase produced by the soil bacterium *B. safensis* could destroy the fungal cell wall, which was a factor leading to the anti-pathogen effect of *B. safensis* [22].

4.1.2. Intracellular Damage

Intracellular damage means that some antifungal substances can inhibit the synthesis of target cell wall components through complex mechanisms, in order to kill target cells or pathogenic fungi by inhibiting cell respiration and the synthesis of extracellular membrane proteins, or interact with pathogenic fungi DNA, thus affecting the physiological functions of pathogenic fungi [46–48].

An important component of fungal cell membrane is ergosterol. Antifungal substances can reduce the expression of genes related to ergosterol synthesis in *C. albicans* and affect the

synthesis of fungal cell membrane. Genome-wide gene transcription analysis shows that surfactin can down-regulate the expression of several genes involved in morphogenesis or metabolism (e.g., glycolysis, ethanol and fatty acid biosynthesis). In addition, the expression of genes related to ergosterol synthesis (ERG1, ERG3, ERG9, ERG10 and ERG11) were down-regulated by surfactin. Surfactin exposure to *C. albicans* could cause physiological effects and affect gene transcription in *C. albicans* [43]. After the lipopeptide AMP-17 treatment, the expression of genes related to ergosterol synthesis (ERG1, ERG5, ERG6 and MET6) were down-regulated through genetic analysis [44]. A lipopeptide C16-Fengycin A isolated from *B. amyloliquefaciens* fmb60 showed significant antifungal activity against *C. albicans*. The changes of cell wall components exposure and the down-regulation of cell wall synthesis-related genes further prove that C16-fengycin A could destroy the cell wall of *C. albicans*, which is due to the fact that this lipopeptides could change the ultrastructure of cells and reduce the hydrophobicity of cell wall [17].

4.2. Inhibition of Virulence Factors

Because of polymorphic, the morphological transformation of *C. albicans* (especially the transformation from yeast to mycelium) is one of the important factors of pathogenicity. If it does not produce mycelium, the virulence of *C. albicans* will be weakened or even non-virulent. The components and processes that affect the pathogenicity of *C. albicans* or promote its infection are called virulence factors, such as the two-phase transition between yeast and mycelium, secretion of adhesion factors, cell surface invasion, biofilm formation and secretion of hydrolases [49–51].

4.2.1. Inhibition of Yeast-Hyphae Biphasic Transition

The hyphal state of *C. albicans* has a stronger ability of tissue invasion and infiltration. Mutant strains that cannot form hyphae usually show weaker virulence in vitro [52]. NRG1 gene can regulate the two-phase transition of *C. albicans*. When NRG1 gene is overexpressed, the morphology of *C. albicans* is yeast under any mycelial induction conditions [53].

Multiples genes could inflect the yeast-hyphae biphasic transition and biofilm formation. By detecting the gene expression of ALS3, HWP1, BCR1, EFG1 and TEC1, the influence of *B. subtilis* on biofilm formation and hyphal formation of *C. albicans* was investigated. *B. subtilis* can reduce 1 log of *C. albicans* biofilm formation, significantly reducing the impact of *C. albicans* morphology of yeast filaments. ALS3 and HWP1 genes were the most influenced of all genes analyzed, and the biofilm of *C. albicans* associated with *B. subtilis* was reduced by 111.1 times and 333.3 times, respectively. *B. subtilis* can down-regulate the expression of ALS3, HWP1, BCR1, EFG1 and TEC1 genes, which are necessary for biofilm formation and filamentation of *C. albicans* [54]. The marine bacteria *B. subtilis* had antifungal membrane effect against *C. albicans*. Microscopic analysis of its metabolite 5HM2F showed a concentration-dependent biofilm inhibition. Real-time fluorescent quantitative PCR showed that ergosterol content decreased and antifungal drug sensitivity increased, and the expression of genes related to drug resistance mechanism was down-regulated. In vivo studies also proved the antifungal efficacy of 5HM2F [32]. By inhibiting the virulence factors of *C. albicans*, *B. safensis* had antifungal activity, which strongly inhibited the biofilm formation and filamentation of *C. albicans*. The mechanism of bacterial anti-pathogens is partly based on targeting fungal cell walls [22].

Some kinds of *Lactobacillus* spp. can also inhibit yeast-hyphae biphasic transition. The effect of filamentation in *C. albicans* was mediated by reducing the expression of filament-related genes (TEC1 and UME6). *Lactobacillus paracasei* can reduce the in vitro filamentation of *C. albicans* by negatively regulating the TEC1 and UME6 genes that are essential for mycelial production. [55].

4.2.2. Inhibition of Adhesion

Adhesion is the beginning of *C. albicans* infection and is a special interaction between the fungus and other microorganisms, medical devices or hosts. Usually, small biomolecules

that promote *C. albicans* adhering to host cells or other cell ligands are called adhesion factors. In addition to adhesion factors, hyphal regulatory proteins usually influence the adhesion ability of *C. albicans* [56].

The lipopeptide of *B. subtilis* AC7 associated with farnesol (the group sensing molecule) can affect the *C. albicans* biofilm formation on the silicone elastomer under simulated physiological conditions, reduce the adhesion of *C. albicans* up to 60%, effectively prevent the initial adhesion of *C. albicans* on silicone and biofilm growth and prevent *C. albicans* medical device-related infections [57]. Lipopeptides C3 has antifungal activity, anti-adhesion and destructiveness, and also inhibits the biofilm formation of *C. albicans* [58]. The micelle solution of biosurfactant and plant natural product terpinen-4-ol (TP) has antifungal and anti-adhesion properties. Biosurfactant enhances the effect of TP as an antifungal and anti-adhesion compound [59].

4.2.3. Inhibition of Pathogenic Fungal Invasion

The invasion of *C. albicans* to host cells plays an important role in the early stage of pathogenesis. ALS3 and SSA1 proteins are two important invasive proteins of *C. albicans*, both of which can influence the invasion process of *C. albicans* [60]. The cyclic lipopeptide biosurfactant produced by *B. amyloliquefaciens* strain AR2 could influence the biofilms of *C. albicans*. The biosurfactant can reduce the mRNA expression of hyphae-specific gene HWP1 and ALS3 without exhibiting significant growth inhibition, which could prevent the invasion of *C. albicans* and the formation of biofilms [61].

4.2.4. Inhibition of Hydrolase Secretion

After *C. albicans* adheres and forms hyphae, the hyphae of *C. albicans* secrete hydrolases that promote the active invasion of hyphae into cells. The hydrolytic enzymes secreted by *C. albicans* include protease, phosphatase and esterase. The metabolite 5HM2F of *B. subtilis* can effectively inhibit multiple fungal virulence factors, such as morphological transformation and secretion of hydrolase (Protease and lipase) [32].

4.2.5. Inhibition of Biofilm Formation

The biofilm is a multicellular complex formed by *C. albicans* attached to the surface of biological cells or non-biological media. Compared with suspended cells, mature capsular cells have stronger resistance to antifungal agents. The biofilm of *C. albicans* can evade the killing effect of neutrophils by not triggering the generation of reactive oxygen species (ROS) and has a strong resistance to body immunity. In addition, yeast cells in the capsule can cause invasive *Candida* infections, and these yeast cells are more cytotoxic. Therefore, biofilm formation is a significant virulence factor. The biofilm formation of *C. albicans* is regulated by transcription factors (BCR1, TEC1, EFGl, NDT80, ROB1 and BRG1). Various stages of biofilm formation, such as adhesion, mycelial formation, synthesis and secretion of extracellular matrix and biofilm resistance, also affect the formation of biofilm. Adhesion factors ALS1, ALS3 and HWP1 affect the biofilm formation of *C. albicans*. Genes or proteins that affect hyphal formation can also affect the biofilm formation ability of *C. albicans*. The extracellular matrix can protect *C. albicans* from the attack of the immune system, and the synthesis of its main component β-glucans can also affect the biofilm formation ability of *C. albicans* [62].

Increasing the level of intracellular ROS will also affect the formation of biofilm. C16-Fengycin A can increase the levels of ROS, which causes intracellular mitochondrial dysfunction. The antifungal mechanism of C16-Fengycin A might also be the accumulation of ROS, which could reduce the formation of biofilms [17]. Some examples of this review also explained that the metabolites produced by *Bacillus* spp. can inhibit the biofilm formation of *C. albicans* [22,32,35,54,57,61].

5. Conclusions

Bacillus spp. has been successfully applied to the control of pathogenic fungi, including in the field of medicine and biological control. Its metabolites play an important role in the prevention and treatment of fungi, including a variety of metabolites in different mechanisms to kill *C. albicans* or inhibit its growth. The purification and identification of metabolites are difficult. The purified metabolic active substances can prevent and control pathogenic fungi from two aspects: inhibiting the fungi themselves and inhibiting the virulence factors. As an opportunistic pathogen, *C. albicans* has a variety of infection mechanisms, especially its mycelial formation, adhesion characteristics and biofilm formation. For this, *Bacillus* metabolites can play a role. Figure 4 shows the main categories, purification and identification technologies and antifungal mechanisms of *Bacillus* metabolites. In the future, the identification of *Bacillus* metabolites and their anti-*C. albicans* research should focus on: (1) Higher precision separation and identification system used to separate metabolites with similar polarity and molecular weight, so that new structures can be identified. Meanwhile, more omics tools could be used for high-throughput separation and screening of the most active components; (2) More microorganisms and metabolites could be combined to investigate the anti-*C. albicans* mechanism of metabolites, and the mechanisms could be further investigated by genomics. (3) *Bacillus* metabolites could be prepared into a variety of forms such as microbial agents or bacterial solution, which is more convenient for the control of *C. albicans* and extended to control more pathogenic fungi.

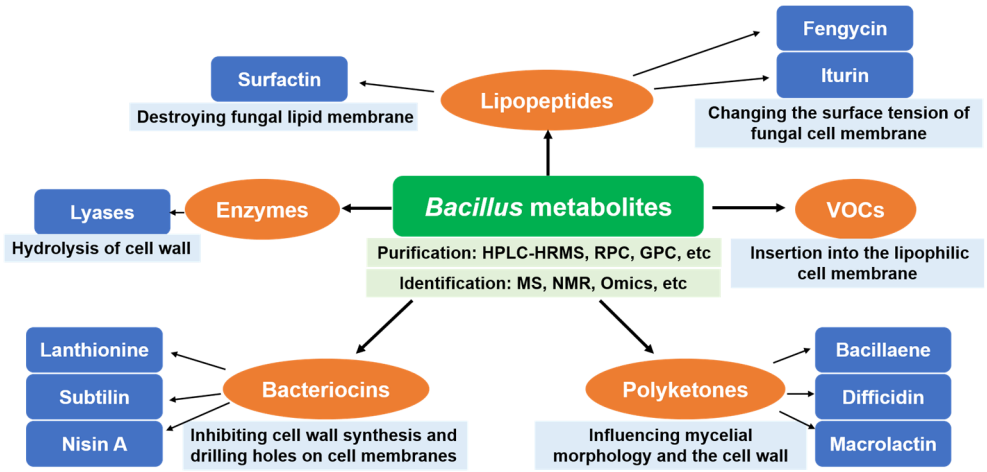

Figure 4. Classification, purification and identification technologies and antifungal mechanisms of *Bacillus* metabolites.

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