Soil *Bacillus*- A natural source of antifungal compounds against *Candida* infection

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Soil, though inert material, posses dynamic and most bio-diverse ecosystem as millions different species of microorganisms dwell in it. The soil ecosystem shows multifaceted interactions and antagonistic activities among microbes which leads to development of various bio-active compounds. These natural agents of biocontrol appear to be potential and reliable substitute for synthetic chemicals. Tropical fungal infection, *Candidiasis* is one of the prevalent infections of humans, affecting various organs like skin, oral cavity & esophagus, gastrointestinal tract, vagina and vascular system and being fatal in immunocompromised patients. The development of resistance to traditional antifungal drugs demands an alternative bioactive compound. Soil *Bacillus* sp. offer several advantages over others for mitigating dermatophytic contagion due to their inherent property producing antibiotics belonging to the family of iturin, mycosubtilin and bacillomycin. Moreover, their ubiquitous occurrence, sporulation potential and solublizable antibiotics make them an appropriate candidate against *Candida* infection. Lipopeptides, oligopeptides synthesized in a non ribosomal manner by large multi-enzyme complexes are the most amphiphilic antimycotic compounds with cyclic structures of β-hydroxy fatty acids or β-amino acids embedded in peptide moiety. The present chapter focuses on antidermatophytic potential of soil *Bacillus* sp. with their physio-chemical action and control against tropical candidal infections.

Keywords: Soil *Bacillus*, *Candida* sp., *Bacillus* metabolites,

1. Background

The global burden of fungal diseases is estimated over 300 million among which 25 million are at edge of dying. Most of the fungal infections, caused due to *Trichophyton, Cryptococcus, Aspergillus* and *Candida* sp., are grouped into filamentous fungi or mould and yeasts (e.g *Candida* and *Cryptococcus*). Warm, moist and dark areas of the body are the usual locations of yeast related infections [1]. Yeasts being commensal in healthy humans, sometimes many of us were unknowingly infected at birth which becomes the basis for an entire lifetime of weakened immune systems and poor health. Around 160 years ago, when patho-physiology of the thrush in newborns was established as the disease due to yeast-like microbes, the infection was considered as trivial which were treatable with simple boric acid (Berg, 1839; Robin, 1853). Since then such infections were commonly named as "thrush" (English), "muguet" (French) or "Soor" (German) indicating global prevalence. Based on population and disease demographic studies (age, gender, immune-compromised etc.), *Candida* species are the most well known fungal pathogens of humans affecting anyone at any age; even in recent era of emerging pathogens.

Unlike the terms “salmonellosis”, “brucellosis” or “tuberculosis” indicate disease state of an individual, the frequently used terms "candidosis", "candidiasis" etc. do not clearly differentiate between the commensal occurrence of *Candida* cells at an absolutely harmless condition and a true infection resulting from its opportunistic exploitation of a set of circumstances with colonization and penetration leading to "Candidamycosis". The term “opportunistic” imply that the infection appears in individuals of compromised immune systems and the pathogen is non-commensal in nature. But both of these implications are misleading as patients who develop Candidal infections typically have specific predisposing risk factors, moreover they do not have always subsided immune systems. Soil being the largest natural source of microorganisms or microbial derived secondary metabolites will be helpful in finding new potential antibiotics. In addition, current regimes of antifungal are not sufficient enough to treat various Candidal infections.

Hence, polypeptide antibiotics from *Bacillus* sp. have been gaining importance because of their diverse antimicrobial activities. The present chapter is pointed out the importance of soil *Bacillus* sp. as the basis of development of potentially active antifungal compounds.

2. Prevalence of *Candida*

Among the estimated total number of 611,000 fungi species around 600 species are pathogenic to humans only [2]. But 99% of all fungal infections are dominated by the less than 30 species fungus. The impact of various mycotic infections, particularly infection due to *Candida*, have increased manifold because of their frequency, occurrence and severity.

Globally the *Candida* is considered as the fourth leading cause nosocomial or health care associated infections with an incidence rate of 8% to 10%. Since 1980, the rate of invasive fungal infections occurrence and prevalence have

increased significantly. The mortality due to systemic candidiasis ranges from 15%- 35% [3]. Given the limitations of blood cultures based diagnosis, the true epidemiology and incidence of invasive candidiasis is very imprecise, even if, the incidence of candidemia can be 8 persons per 1 million [4]. Apart from immunocompetent individuals, the prevalence and effects of *Candida* sp. on immunocompromised individuals are very significant with higher numbers of morbid and fatal individuals [5]. Therefore, it is most often regarded as "disease of diseased".

2.1 Types of Candida

The genus *Candida* is a heterogeneous group, comprising of approximately 150 disparately related species that grow mainly as unicellular yeasts, but sometimes have the ability to grow in pseudohyphae and hyphae (e.g., *Candida albicans*) forms. The global prevalence data collected over 10-years period (1997–2007) from 142 institutions in 41 countries (e.g. SENTRY and ARTEMIS) have identified 31 species of *Candida* in clinical samples [6]. However, only 15 to 24 species are etiological agents of human infections which include *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. guilliermondii*, *C. lusitaniae*, *C. dubliniensis*, *C. pelliculosa*, *C. kefyr*, *C. lipolytica*, *C. famata*, *C. inconspicua*, *C. rugosa*, and *C. norvegensis*. The following five species, *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*, are predominantly isolated and responsible for just over 92% of cases invasive infections and 13 other *Candida* sp. were rarely isolated (incidences of 0.01%) [6].

The *C. albicans*, the main representative of this group of imperfect, i.e. anascosporogenous budding fungi is distributed as a usual flora of mucous membranes of healthy individual like mouth, gastrointestinal and urogenital tracts. With the favourable predisposing factors, *C. albicans* infection can manifest in other parts of the body. The *Candida* species specific global distribution and incidence of candidal infections (*C. albicans* versus non-albicans *Candida* (NAC) spp.) differ among regions to health-care setup or even among patient cohorts. For instance *C. guilliermondii* and *C. rugosa* are more prominent in Latin America and *C. inconspicua* and *C. norvegensis* in Europe [6]. In recent years, various studies highlight an increase of NAC, such as, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. dubliniensis* [5]. While NAC species accounted for 10%– 40% of all systemic candidiasis in 1970-90 which was reached 35%–65% in the last two decades [7]. The *C. parapsilosis* causes 30% of the candidemia cases among newborns whereas the rate is 10%–15% in adults. Similarly, while *C. glabrata* being more common among older and neoplastic patients *C. tropicalis* affects more leukemia as well as neutropenic patients. However, *C. krusei*, is common in hematopoietic stem cell recipients. A comprehensive list of *Candida* sp. and their site of infection is provided in Table 1.

2.2 Pathogenicity and predisposing factors of fungal infection

Fungal infections of *Candida* sp. can be broadly categorized into three major groups: (i) cutaneous, the infection of skin and its appendages (mostly affects AIDS patients); (ii) mucosal (oropharyngeal, esophageal and vulvovaginal infections observed in immunocompetent infants and adult women) (iii) invasive candidiasis (IC) which includes candidemia, endocarditis, disseminated infections, central nervous system infections, endophthalmitis and osteomyelitis, observed in cancer or immunosuppressed patients. It is estimated 80-90% of HIV patients are infected with oropharyngeal candidiasis (OPC), at least some point of their during disease progression [8]. Approximately 75% of all women suffer at least once in their lifespan from VVC [9].

The common clinical risk factors for mucosomal and disseminated candidal infections involve disruption of normal ecological and anatomical barriers which separate external compartments colonized by *Candida* from aseptic internal tissue and blood. Nylon fabric based tight clothing can trap heat & moisture which provide favorable environment for yeast infection. One of the major factors of proliferation of *Candida* is based on the fact of indiscriminate use of broad spectrum antibiotics which militate against susceptible microflora antagonistic to the fungi [10]. Traumatic ulceration, postoperative situations, malnutrition, malignancy and anaemia also predisposes candidiasis. The occurrence of *Candida* in diabetic patients may be attributed to high level of blood sugar providing rich energy source. Other predisposing factors for proliferation of *Candida* are pregnancy, administration of corticosteroids or immunosuppressive drugs, drug addiction and immunological deficiencies. Besides, systematic conditions such as vitamin B deficiency, hypothyroidism and lymphoblastoma [11] also favor the multiplication and turning of harmless commensal to a noxious opportunist which becomes invasive and fatal to its victim.

The pathogenicity of *Candida* sp. is attributed to various virulence factors, such as the ability to evade host defences, adherence, biofilm formation and the production of tissue hydrolytic enzymes such as proteases, phospholipases and haemolysin [12]. Ability of the microorganism to adhere onto the epithelial cells play a major role in the pathogenesis of *Candida* which is carried out protein-polysaccharide interaction like protein-adhesins of the yeast cell binding to the carbohydrates of the host cell. A fungus giving rise to a mycosis must be able to damage tissues by secretion of extracellular enzyme located on cell surface of hyphal tip. During the invasion, a morphological alteration (formation of mycelia) takes place and the well known dimorphism of *C. albicans* is expressed. In tissues, the free iron has less availability as it is being complexed with iron-binding proteins. Therefore, pathogenic microbes have evolved siderophores, to compete successfully for iron. Thymopentin controls the early T-cell differentiation and maturation,

essential for cell mediated immunity. So any, T-cell disorders are predisposed to chronic mycoses like chronic mucocutaneous candidosis or chronic recurrent vaginal candidosis.

Table 1 Prevalence of Candida species with infecting area and response to drugs

Sl.	Organism	Infecting Area of Body	Effected Country	Resistance to Drugs
1	*C. albicans**	Oral mucosa, Gut, Vaginal mucosa, Skin, Blood, Heart	World Wide	Flucanozole, Caspofungin, Flucytosine
2	*C. glabrata*	Oral cavity, Systemic blood infection, Urinary tract	USA, Can, Eur, Lat. Ame.	Micafungin, Anidulafungin, Flucanozole, Caspofungin, Polyene
3	*C. parapsilosis**	Skin, Nail, Hands, IV catheters, Blood	Asia, Lat. Ame, Eur, N. Ame	Amphotericine B, Voriconazole,
4	*C. tropicalis**	GI Tract, Blood stream	Asia pacific, N. Ame, Lat Ame, Eur, Ind	Flucanozole, Voricanozole, Caspofungin
5	*C. krusei*	GI Tract	Asia pacific, N. Ame, Lat Ame, Eur	Amphotericine B, Voriconazole, Flucanozole
6	*C. dubliniensis**	Oral cavity, Oropharygeal cavity	World Wide	Amphotericin B, Flucanozole, Echinocandines
7	*C. gulliermondi**	Nail infection, Invasive candidasis	Lat Ame, N. Ame, Ind, Eur,	Flucanozole. Echinocandins
8	*C. lusitaniae**	Blood, Invasive cathedral	Asia pacific, N. Ame, Lat Ame	Amphotericin B,
9	*C. rugosa*	Blood, Urinary tract, Implant	Asia pacific, Lat Ame Eur	Flucanozole, Nystatin, Apmhotericine
12	*C. famata*	Blood stream	-	Echinocandins, Flucanozole, Flucytosine
13	*C. incospicua*	Oropharengeal cavity, Vaginal lining	-	Flucanozole
14	*C. kefyr*	GI Tract, Oral cavity, Urinary tract	-	Amphotericin B, Echinocandin
15	*C. lipolytica*	Blood stream,	-	Flucanozole
16	*C. norvegensis*	Oropharengial cavity, Abdominal cavity	Eur, Jap	Flucanozole
17	*C. sake*	Oral cavity	Eur	Azole compounds
18	*C. zeylanoides*	GI lining, Blood, Vaginal cavity	Eur	Flucanozole, Ketonazole
19	*C. auris*	Urinal discharge, Respiratory tract	Asia, Lat Ame, USA, Kor, UK	Flucanozole, Vericunazole, Amphotericin B, Echinocandin
21	*C. fermentati*	Eye	Ind	Voriconazol, Itraconazole, Flucanozole
20	*C. pelliculosa*	Blood, Urinary tract infection	Brazil	Flucanozole, Itraconazole, Ketonazole, Glucytocine
22	*C. palmioleophila*	Blood	Denmark	Itraconazole, Voriconazole
23	*C. haemilonii*	Blood, Toe nail	Eur, S. Kor, Lat Ame	Amphotericine, Azole comp
24	*C. africana*	Female Genital Specimen	UK, Africa, USA	Flucytocine, Voriconazole, Terbinafine

Can: Canada; Ind: India; Lat Ame: Latin America; N. Ame: North America; Pak: Pakistan; SA: South America; S. Ko: South Korea; USA: United States of America; UK: United Kingdom;

GI: Gastro intestinal; IV: Intravenous

Gen: Genomic; *: belong to CTG clade; 1-4:Common; 5-9:Infrequent; 12-24:Rare

2.3 Fungal Drugs

The cell wall of yeast cells, composed mainly of glucan, mannan and chitin which maintains its structure and properties is the target of many antifungals. Ergosterol, instead of cholesterol, is the major sterols in *C. albicans* and other pathogenic fungal cell membrane and striking difference from mammalian membrane. Drugs like allylamines, morpholines and pyridine, pyrimidine, imidazole and triazole derivatives disrupts sterol biosynthesis, localized at the

smooth endoplasmic reticulum. Other important targets are DNA or RNA synthesis, oxidative phosphorylation and the ATPases in mitochondria. Similarly, Griseofulvin targets the microtubules of fungal cells. and plasma membrane

2.4 Failure of drugs

The modern pharmaceutical practices visualizing fungi/yeast as serious challenge to conventional drugs. These drugs not only have side effects but also current regimes of antifungals are becoming slowly ineffective. The ability of *Candida* sp. to form drug-resistant biofilms is the driving factor fungal pathogenicity. The generic fungistatic azoles drugs primarily act on ergosterol biosynthesis. The modification of the target protein or the over expression of gene represent commonest mechanisms of multidrug resistance (MDR) where the target protein of azoles, Erg11p, is modified by the mutations replacing the native amino acids. In addition to this, ERG3 alone or in combination with ERG11 mutations, result in change in the ratios of various cell sterol biosynthetic intermediates and increased tolerance to azoles and polyenes. The property of resistance (multidrug) is achieved by overproducing the plasma membrane pump proteins that efflux the drugs from cells. This is evident from the fact that protein-pumps like ATP Binding Cassette (ABC) multidrug transporter proteins (Cdr1 and Cdr2) or major facilitator superfamily (MFS) efflux pump protein, Mdr1, are over-expressed in azole resistant clinical isolates [13]. Therefore, increase in resistant strains necessitates a search for newer antifungal agents.

3. Soil microbes- the natural source of antimycotic compounds

In spite of being familiar and useful, soil is also one of the least understood habitats on earth as the most important natural resource which directly or indirectly favours the growth of almost all land life forms. Soil is a composite environment as well as stockpile of microbial genetic diversity. The microbial diversity in soils outdo that of other environments as 1 gm of soil contains up to 10 billion microorganisms of possibly thousands of different species. Soil microorganisms have been continuously screened for their useful bioactive metabolites such as antibiotics long back. Soil bacteria show antifungal properties due to the production of different enzymes which may be a part of their lytic system that enables them for living on hyphae or pseudohyphae as appropriate substrate for growth [14]. It is, however, arguably still true: comparisons of the information presented on sources of new drugs since 1981 to 2007 indicate that almost 50% of the drugs approved since 1994 are based on microbial natural products. Most natural products such as antibiotics or other pharmaceuticals have revealed tremendous chemical virtuosity, utility and economically viable and are derived from cultured soil microbes [15].

Many bacterial species are known to produce antimicrobial agents. These could be bacteriocins, inhibitory proteins or peptides [16]; cationic peptides, glycopeptides, ß-lactams, quinolones, streptogramins, glycylcidenes, ketolides and oxazolidinones [17]. Regardless of the toxicity of few antibiotics synthesized by bacteria from soil *Bacillus* sp. to the cells of mammals (e.g. polymyxines, bacitracin, etc.), they were and continued to be in the focus of attention of scientists. Lipopeptide surfactin was also exerts antifungal activity such as formation of membrane ion channels. The antifungals of microbial origins have following advantages: high specificity against target fungal or yeast pathogens; easy degradability and paves way for mass and rapid multiplication which could be utilized for its proper economical return.

Out of 100 soil bacterial isolates from Egypt, 20 could antagonize some selected human and plants pathogenic fungi such as *Aspergillus* sp., *Fusarium oxysporum*, *Alternaria solani* and *Penicillium digitatum* [18]. Twenty bacterial strains isolated from stressed soil of Eastern Uttar Pradesh, India exhibited strong antimicrobial activities [19]. *Bacillus subtilis* from dicerse environment such as acid soils expressed considerable activity against human dermatophytes [20]. A few saprotrophic soil bacteria (*Actinobacillus* sp., *Clostridium* sp., *Streptococcus* sp.) can serve as excellent biocidal agents against human pathogens [21]. *Streptomyce*s sp., another spore former produces bioactive compounds which can be formulated readily into stable products have been used for antibiotic production for more than a quarter ton. They contribute to 75% of the identified products which are widely used in clinical applications [22]. *Rhodococcus* sp. Gram positive soil bacteria has also been identified to have high potential of producing useful antibiotics under stress condition. Rhodostreptomycin, the amino glyceride antibiotic from *Rhodococcus* sp. is effective against many types of microorganisms [23].

4. Soil *Bacillus*- The potential source

Soil as the prosperous resource of microorganisms, a wide range of natural antibiotics of peptidic nature have been produced. These metabolites have played a key role in the discovery and development of various antifungal antibiotics. The soil microbial isolates exhibiting antimicrobial activity under natural conditions and was found inhibiting various pathogenic yeast and molds [24]. Naturally the antibiotics secreted by different microorganisms are responsible for the regulation and control of microbial population to successfully colonize lithospheric niche. Among the soil bacterial diversity as bacillus (rods), coccus (spherical or rounded) and spirilla (spirals) of which, *Bacillus* are more frequent and

widely distributed [25]. *Bacillus* sp. bears gram positive, sporulating, chemoheterotrophic, motile (peritrichous flagellar movement), aerobic or facultative anaerobic physiochemical characteristics [26].

Many investigations have been carried out to isolate various strains of *Bacillus* from soil and identify their bioactive metabolites [27]. At present, soil *Bacillus* produces bioactive metabolites of more than 200 nos. of different types and is viewed as a promising point in the search for new bioactive substances [28]. Out of these *B. subtilis* as an important producer and secretes more than 70 nos. of metabolites while *B. brevis* produces approx. 30 nos. Most of the *Bacillus* sp. are considered as safe and possess inheritable capacities to synthesize many substances, several lipopeptide compounds, a class of microbial surfactants that have been successfully used in healthcare and allied realm [29]. The secondary metabolites exerts an array of biocidal activity. The soil *Bacillus* represents a vast range of physiological abilities and adapted to grow in extensive range of soil environments with varying salt concentrations, temperature & pH. *Bacillus* antibiotics are usually produced at the early stages of sporulation while in some species sporulation had less effect on antimicrobial substance secretion. Production of active metabolites is reported to be dependent on available nutrients, enzyme inactivation process and growth period/phase [30].

4.1 Overview of antifungal compounds from soil *Bacillus*

Bacillus-derived bioactive natural products as establish a pool of compounds with a large and indulged structural diversity, showing an array of bioactivities such as antibacterial, antifungal, antiviral, antitumor, antiamebocytic and antimycoplasmic properties. The recognized *Bacillu*s species that produce antifungal antibiotics are *B. subtilis*, *B. brevis*, *B. licheniformis*, *B. circulans*, *B. cereus* [31]. Since more than a half century peptide antibiotics representing the predominant class produced in *B. subtilis* were reported to inhibit several clinical fungal pathogens along with some food spoiling bacteria due to varying potencies [32]. The peptide antibiotics basically lipopeptides or polyketides are highly heat (≥100 °C) stable, pH-tolerance and resistant to proteolysis [33]. The majority of peptide antibiotics like bacillomycin, mycobacillin and fungistatin are able of targeting and disintegration of fungal cell walls [34].

Few strains of *B. amyloliquefaciens* produce both peptides and non-peptide metabolites that can help in inhibition of pathogens. Moreover, if the compounds are novel and safe, they can be good candidates for the development of new drugs [35]. A recent study showed that iturin can be used for biological control of *C. albicans* including several other human important fungal pathogens [36]. Both the *B. subtilis* and the *B. mojavensis* have a potential of lipopeptides and cell-wall degrading enzymes production, which acts as an alternative resource for control of *C. albicans* [37]. Two *Bacillus* strains (CWSI-B1567 and CWSI-B1568) from arid region soil secreted metabolites to act as antifungals against *C. albicans* the causative agent of candidasis, both *in vitro* and *in vivo*. Surfactin, a 7-amino-acid residue lipopeptide antibiotic produced by *B. subtilis* acting against fungal infections. Tyrocidine and Gramicidin S are cyclic peptides produced by the *Brevibacillus brevis* (erstwhile *Bacillus brevis*). The gramicidin S synthetase a multifunctional enzyme complex catalyzes Gramicidin S synthesis [38]. Subtilin, an antifungal compound is produced from *B. subtilis* along with several other antibiotics as subsporins A–C, lipooligopeptides; and rhizocticins A-D, phosphooligopeptides [39].

B. cereus and *B. licheniformis*, excreated several antifungal compounds, mainly lipopeptides, have also been successfully tested against candidiasis [40]. In some instances the production of active metabolites (lipopeptides) allows certain strains of *B. subtilis* to modify cell wall and to regroup together in a biofilm in order to proliferate and spread in the territory [33]. *B*. subtilis produces lipopeptide bacillomycins L, D, bacillopeptins, rhizocticin A, a hydrophilic phosphonooligopeptide active against *Candida* sp. *B. cereus* produces cispentacin, active on *C. albicans* both *in vitro* and *in vivo* in mice, and mycocerein, an iturin antifungal, which inhibits growth [41].

4.2 Structural and functional aspects of antifungals

Most of the bacteria produces antibiotics as secondary metabolites via enzymatic catalysis involving a series of biosynthetic pathways. Antimicrobial peptides are considered the primordial in invaded eukaryotic and prokaryotic cells [42]. Based on origin or synthesis antimicrobial compounds from *Bacillus* sp. are divided in to two types. One is ribosomally synthesized followed by post translationally modification and other nonribosomally synthesized such as lipopeptides or polyketides and a phospholipid [29] via multistep mechanism(s) which involves active participation of multi enzyme thiotemplates. Due to involvement of multienzyme complexes with modularly arranged catalytic domains [43] the nonribosomal antibiotics exhibit a broader specificity than ribosomal counterparts. This synthesis is mediated by nonribosomal peptide synthetases or polyketide synthases pathway employing multienzymes complexes of 100 to >1600 kDa [44]. Apart from this the antimicrobial compounds produced by *Bacillus* are also phenolic derivatives.

Bacteriocins and bacteriocin-like inhibitory substances (BLIS) are ribosomally synthesized peptides, Cerecin 7, Tochicin, Thuricin7, thuricin439 and entomocidus 9, sublancin, subtilosin A, TasA polymyxin, difficidin, subtilin, mycobacillin, bacitracin, barnase, etc. are few of them and exhibited very less antagonistic effect on human pathogenic yeast and mould [45].

Amongst all, nonribosomal peptide antibiotics are the prevailing class of antimicrobial molecules, synthesized by *B. subtilis* [29]. It includes small lipopeptides(<2000 Da) as surfactin family: surfactin and lichenysins [46]; iturin family: iturin A, D and E, bacillomycin D, F and L and mycosubtilin [47]. Synthesis clusters of bacillaene, bacitracin, difficidin, iturins (bacillomycins), macrolactin, surfactin, bacillibactin, bacilysin a dipeptide also have been detected in

B. subtilis. In other words, iturin A belong to cluster 1 which contributes to the antifungal activities of *B. velezensis* (erstwhile *B. amyloliquefaciens*) strain CC09 [48]**.** Iturins consist of a peptide ring of seven α-amino acid residues, with the constant chiral sequence LDDLLDL and common presence of D-Tyr2, closed by a β-amino fatty acid with 14 to 17 carbon atoms [49]. Subtilin, a 32-residue peptide produced from *B. subtilis*. Further, these can be modified by N-methylation, acylation, glycosylation or heterocyclization [50]. Apart from that polypeptide antibiotics produced by *Bacillus* that are of pharmaceutical importance and used in medical treatments are gramicidin J/S, tyrotricidin [51]. In comparision to Iturins and fengycins, surfactins are not fungitoxin by nature but able to do so via synergism with Iturin A [49]. Surfactin has exceptional surfactant activity and emulsification properties. The active compounds are produced by *B. subtilis* and *B. tequilensis* also exhibit stability to heat, pH and proteolytic enzymes, which widen its potential industrial importance [52]. Among the lipopeptides, iturins and others as in Table 2 have shown antifungal activity, simultaneously, surfactin showed less marked fungitoxicity [46].

It is pertinent to mention here that lipopeptides from *Bacillus* made of amino acids and a fatty acid, are amphiphilic membrane active biosurfactants. Each family contains variants with the same peptide length but with differ in residues at different positions. Moreover, each variant can have several homologues of different length and isomer of the fatty acid chain, leading to a remarkable structural heterogeneity [46]. All these amphipathic cyclic biosurfactants have high biodegradability, low toxicity and ecofriendly characteristic advantages.

4.3 Mode of Action of antifungal compounds

The peptide antibiotics can inhibit peptidoglycan synthesis using some specific molecules such as the mannose phosphate transferase system (man-PTS) or lipid II as a docking molecule [53]. Lipopeptides are metabolites that can function as a pore formation or emulsification on the target organism. Data from scientific research opined that iturin forms pores that increase the ion conductance of fungal outer lipid membranes. It is also known to disturb the cytoplasmic membranes of yeast cells, causing leakage of monovalent potassium ions as shown in Fig 1 and other vital constituents in leading to death of yeast cells [46, 49]. The fatty acid moiety of Iturin family lipopeptides (e.g., iturin A, bacillomycin D/F) contain β-hydroxy fatty acids with a 14-carbon chain ceases growth of different fungi. Mycosubtilin (iturin family) secreted produced by *B. subtilis*, strongly active against different yeast species [54]. Further studies on fungal membranes and mycosubtilin showed that the action of lipopeptides was dependent on the reciprocal actions with phospholipid and sterols, especially with the acyl trails of the phospholipid and alcohol group of membrane sterols [55].

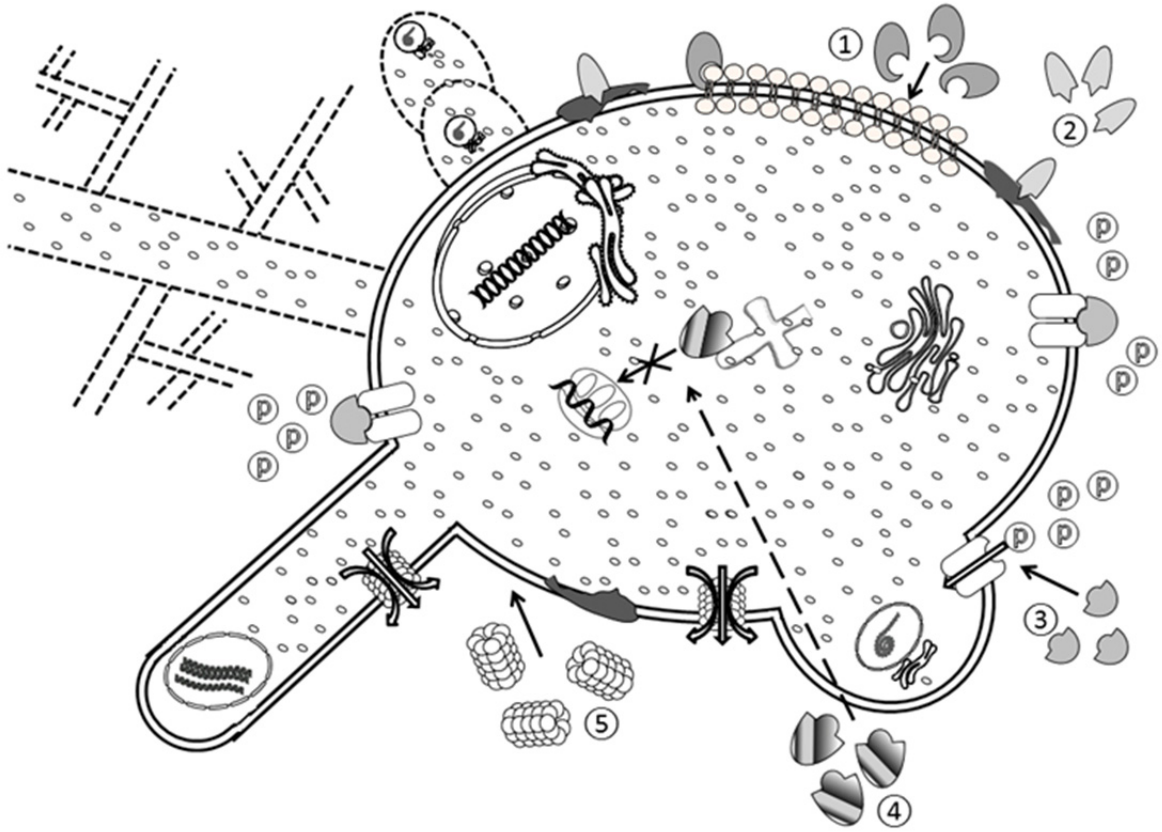

Figure 1 Prospective mechanism bacillus inhibitory compounds; 1.Phospholipase activation, to release of phospholipids and others; 2.Cell surface protein disruption; 3.Blocking the amino-acid and ion uptake; 4. t-RNA inhibition; 5. Cell surface pore formation

Table-2 Different soil *Bacillus* compound active against different *Candida* sp.

Soil *Bacillus* sp.	Pathogenic *Candida* sp.	Antifungal Compound	Mode of action
B. subtilis	*C. albicans*	Mycobacillin	Growth inhibition through cell agglutination
B. subtilis	*C. albicans*; *C. glabrata;C. tropicalis*	Datemycin	Agglutination of mycelium
B. subtilis; B. amyloliquefaciens	*C. albicans*	Surfactin	By forming ion conducting membrane channels in cell walls
B. subtilis	*C. albicans*	Pseudofactin	reduction in cell surface hydrophobicity via β-1,3-glucan degradation
B. subtilis	*C. albicans;C. glabrata;C. tropicalis*	Mycosubtilin	Formation of transmembrane pores to disrupt cell membrane
B. subtilis;*B. licheniformis*; *B. pumilus*	*C. albicans*	Bacilysin	Inhibition in peptide transportation
B. amyloliquefaciens; B. pumilus	*C. albicans*	Anticapsin	Inhibition of the NH_4^+ uptake prohibiting amino-acid synthesis
B. subtilis	*C. albicans*	Bacillomycin D	Formation of pore in cytoplasmic membrane
B. subtilis	*C. albicans*	Bacillomycin F	-
B. subtilis	*C. albicans* & others	Bacillomycin L	Phospholipase activation, to release of phospholipids and others
B. amyloliquefaciens	*C. albicans;C. tropicalis*	Lipopeptides	Induces membrane permeability
B. licheniformis	*C. albicans*	Lichenysin A	Inhibition in biofilm formation
B. licheniformis	*C. albicans*	Lichenysin B	Reducing interfacial tension and reducing interaction
B. subtilis	*Candida* sp.	Iturin A	Leakage increase of K^+ ions and other vital constituents
B. subtilis	*C. albicans*	Iturin D/E	-
B. cereus	*C. albicans;C. tropicalis;C. krusei*	Cispentacin	Blocking Aminoacyl tRNA-synthase for amino acid synthesis
B. cereus	*C. albicans;C. glabrata; C. kefyr*	Azoxybacilin	1. Inhibit germ tube formation 2. Preventing S containing a.a. synthesis by homoserine dehydrogenase inhibition
B. subtilis	*C. albicans*	Rhizocticin A	Blocking peptide transportation for threonine or related metabolism
B. megaterium	*C. albicans*	Bacimethrin	Inhibits thiamine and pyridoxine(aminoacid) synthesis
B. aneurinolyticus	*C. albicans*	Tyrothricin	Inhibits amino acid synthesis
B. brevis	*C. albicans*	Gramicidin J/S	Destroyes Ionophors and enhances K^+ release
Bacillus sp.	*C. albicans*	Iturin(type compounds)	-

-Not known; S= Sulfur; a.a.= amino acid

Tyrocidines are cationic cyclodecapeptides significantly produced by *Bacillus aneurinolyticus* have effective successfully against *C. albicans* at low quantity (μM range). It disrupts the membrane integrity by rapid lysis of membranes composed of ergosterol and phosphatidylcholine results in prevention of *C. albicans* biofilm formation *in vitro*. Tyrothricin, a secondary metabolite peptide complex, formed by the combinations of tyrocidines and gramicidins, produced from *B. aneurinolyticus* [56]. Tyrocidine activity induced ROS formation by membrane disruptive activity in a concentration-dependent manner. The depletion of *C. albicans* membrane integrity is probable due to direct peptide-membrane activity, limiting resistance potential resulting in decreasing cell viability [57]. WH1fungin, a new cyclic lipopeptides member of the surfactin family produced by *B. amyloliquefaciens* WH1. The mechanism of action includes two steps. First it creates pores in the cell membrane at higher concentration and secondly it induces apoptosis at lower concentration. It prevents glucan synthase, resulting in decreased callose synthesis in the fungal cell wall. Additionally, it has been found that this lipopeptides attaches to an ATPase on the mitochondrial membrane and reduces its activity in fungal cells. In addition to this it also targets fungal genome [58].

Current combat against candidiasis include a variety of azoles that inhibits the synthesis of ergosterol, polyenes aiming to bind fungal membrane ergosterols for dysfunction. The echinocandin lipopeptides that inhibit (1,3)-glucan synthase activity, resulting in fungal cell death [59, 60].

4.4 Genomics of antifungal soil *Bacillus*

The surfactin biosynthesis in *B. subtilis* is regulated by srf A operon. The gene *sfp* encoding a 4'-phosphopantetheinyltransferase and the comA regulatory gene work altogether for the biosynthesis. Srf A operon spans 25 kb is responsible for sporulation and competence development. It contain 4 nos. of modular Open Reading Frames (ORFs), ORF1 (srfA-A), ORF2 (srfA-B), ORF3 (srfA-C) and ORF4 (srfA-D) organized into 7 amino acid-activating domains. The *sfp* gene, mapped at 4 kb downstream of the srfA operon and associated but not integrated in the latter, is the second gene essential to produces surfactin [43].

The gene cluster from *B. subtilis* ATCC6633 carries the information specifying for the biosynthesis of mycosubtilin. The putative operon is of 38 kb long and contains four ORFs, designated mycA, mycB, mycC and fenF have strong homologies to peptide synthetases. Biochemical characterization showed that adenylates tyrosine (broadly MycB), required for mycosubtilin synthetase enzyme leads to mycosubtilin production [61]. Moreover, Bacillomycin D operon, 37.8 kb long with four ORFs: bamD, bamA, bamB and bamC and similar to the organization(structural) of the Mycosubtilin and Iturin A operon [62]. The gene bacABCDE (=ywfBCDEF) sequences responsible for biosynthesis of bacilysin has been identified from *B. subtilis* genome. Amplification of the bacABCDE gene cluster in a bacAB deficient *B. amyloliquefaciens* strain resulted in a tenfold bacilysin overproduction [63]. However rhizospheric soil isolate *B. amyloliquefaciens* dedicated 8.5% of genome for non ribosomal secondary metabolites synthesis [64].

5. Potential and spread of antifungal compounds

Despite extensive scientific findings for the development of new therapeutic strategies, there are only a few availed drugs for combat on *Candida*. Prolific research indicating the potential health benefits and spread of *Bacillus* metabolites for use. Indeed, only few molecular classes targeting approx. five distinct fungal metabolic pathways are currently used in medications for systemic fungal infections. These are as fluoropyrimidine analogs, polyenes, azoles and echinocandins [65]. However, the efficacy of some of these synthetic drugs is severely limited because of their poor activity in blood, the emergence of resistance and unacceptable toxicity; thereby underscoring an urgent necessity for new antifungal agents of environmental friendly required. Unfortunately, the development of an entirely new drug is a long and expensive process. New drugs have to undergo an arduous approval process by the USFDA in order to establish safety of the drug for human consumption [66]. Overall, all compounds discussed in this chapter are basically divided into three structural categories: five membered heterocyclic drugs (azoles and oxoles), six membered heterocyclic compounds (pyridines) and other structures. It is found that the compounds belong to *Bacillus* sp. and synthesised non ribosomal lipopetides are effective in preventing *Candida* sp. Basically they could be used is drugs of three different chemical classes: azole, oxole and other structures. Thus, it could be purposed new for antifungal drugs of microbial origin. With the potential to be one of the safest of the developed drugs as it is insensitivity of mammalian cells because and do not affect the sensitivity of mammalian cells significantly [67].

6. Future prospects

Various papers have reported the diversity in pathogenicity, clinical manifestations, prevalence and mycological aspects; the candida infections or candidiasis or candidamycosis cannot be treated as one entity rather the etiologic depend completely on several parameters. Much emphasis has been made to understand pathogenesis. Molecular techniques, rec-DNA Technology and proteomics research have been helpful in understanding the diversity and

exploring the intricacies of cellular pathways of candidal infection. Soil microbes and secondary metabolites produced by them have played a significant role in the upgradation as well as discovery of many antibiotics. Members of the genus *Bacillus*, proliferative group of soil bacteria, produce compounds that may contribute to development effective antifungal drugs. Understanding diversity patterns, identification and isolation of new bioactive compounds and mode of action of microbial metabolites will propel pharmaceutical industry. Few studies attempt to determine quantitative activity of bacilli in soil, or the specific processes which they carry out, in relation to the activities of the total microbial community. Once more, we strongly advocate expanding, not decreasing, the exploration of nature as a source of novel bioactive agents which may serve as the leads and scaffolds for extensive elaboration into seriously needed efficacious drugs for a series of disease indications.

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