Int.J.Curr.Microbiol.App.Sci (2018) 7(2): 823-835

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 02 (2018)**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2018.702.104

Bacillomycins – The Effective Molecules in Plant Disease Management

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Keywords

Bacillomycins, Molecules, Plant disease management

Article Info

Accepted:
10 January 2018
Available Online:
10 February 2018

ABSTRACT

Bacillomycins are the cyclic lipopeptides belonging to the iturin family produced by *Bacillus* spp. mostly *Bacillus subtilis*, which are biosynthesized by multimodular proteins termed as NRPSs (non-ribosomal peptide synthetases) genes. There is tremendous research on *Bacillus* spp. lipopeptides or antimicrobial peptides (AMPs) showing great antagonistic potentiality of these strains, especially with bacillomycins producing genes. This review highlights the bacillomycins structure, biosynthesis, mode of action and the noteworthy antagonistic *Bacillus* strains with bacillomycin producing genes which have the huge potential of commercialization of these antibiotics. Moreover, an outlook is also given in a view of commercializing potential of bacillomycins against plant diseases, though there are commercial applications of bacillomycins in bio-pharmaceutical, food, textiles, pulp/paper & drug industries.

Introduction

The ubiquitous *Bacillus* spp. with great genetic and metabolic diversity leads to the production of several antibiotics and enzymes which has increasing demand in biotechnological approaches and plant protection. Several hundred wild-type *B. subtilis* strains have been collected, with the potential to produce more than two dozen antibiotics with an amazing variety of structures. All of the genes specifying antibiotic biosynthesis combined amount to 350 kb; however, as no strain possesses them all, an average of about 4–5% of a *B. subtilis* genome is devoted to antibiotic production (Stein, 2005).

The potential of *B. subtilis* to produce antibiotics has been recognized for 50 years. Peptide antibiotics represent the predominant class. In the context of biocontrol of plant diseases, the three families of *Bacillus* lipopeptides – surfactins, iturins and fengycins were at first mostly studied for their antagonistic activity for a wide range of potential phytopathogens, including bacteria, fungi and oomycetes (Ongena and Jacques, 2007). Among them, Iturin is a large family of cyclic lipopeptides produced by *Bacillus* sp. It consists of iturin A, iturin C, iturin D, iturin E, bacillomycin D, bacillomycin F, bacillomycin L, bacillomycin Lc and mycosubtilin (Peypoux *et al.,* 1973, 1978, 1979, 1984; Besson *et al.,* 1976; Besson and Michel, 1986;

Cho *et al.,* 2000; Yu *et al.,* 2002). This review highlights the importance of cyclic lipopeptide, Bacillomycins of Iturin family produced by various species of *Bacillus* (especially by *Bacillus subtilis*) in wider environment, its isolation, biosynthesis with a keen view on biosynthetic gene clusters studied in various strains of *Bacillus*, molecular method of detection, mode of action and gathered the works on antagonism of *Bacillus* spp. with bacillomycin producing genes against plant pathogens which also involves the attempt to commercializing production methods of the particular antibiotic, bacillomycin.

Bacillomycins

The first form of bacillomycin isolated from *Bacillus subtilis* strain was Bacillomycin D a new antibiotic of the iturin group was described by using mild acid hydrolysis and by cleavage of the molecule with N-bromosuccinimide (Peypoux *et al.,* 1973, 1981). Then structure of bacillomycin L came into light which is a cyclic lipopeptide from iturin group characterized by a liposoluble ß-amino acid linked to a peptide moiety containing D and L ɑ-amino acids (Besson *et al.,* 1977, 1978). The complete structural analysis of peptidolipid antibiotics, Bacillomycin D and Bacillomycin L isolated from *Bacillus subtilis* was done by Peypoux *et al.,* (1984). Similarly, Bacillomycin F a new antibiotic of Iturin group was also first reported from *Bacillus subtilis* (Peypoux *et al.,* 1985). Another modified form of antifungal antibiotic of the iturin class, Bacillomycin Lc was isolated from a strain of *Bacillus subtilis* as a set of 5-congeners by using chemical and spectrometric analyses (Eshita *et al.,* 1995).

Structure of bacillomycins

The general structure of an iturin is composed of heptapeptide with a conserved chiral sequence LDDLLDL which is cyclized by amide bond formed between α–COO group of 7th amino acid and ß–NH2 group of ß- amino fatty acid (ßAA), which in turn is peptide bonded through its α-COO group to N-terminal amino acid (Fig. 1). The members of iturin family exhibit heterogeneity at 1, 4, 5, 6 and 7 amino acid position/s in the peptide moiety as well as in the ßAA length, which varies from 14 to 17 carbons. On the basis of variation of amino acids in peptide moiety, bacillomycins have been classified as: bacillomycin D, bacillomycin F, bacillomycin L and bacillomycin Lc (Fig.1) (Peypoux *et al.,* 1978, 1984; Besson *et al.,* 1976; Besson and Michel, 1986).

Bacillomycin D is a mixture of two homologous lipopeptides: the lipid moiety consists of 3-amino-12-methyltridecanoic acid or 3-amino-12-methyltetradecanoic acid; the peptide moiety contains one residue of each of the following seven amino acids: L-Asn-D-Tyr-D-Asn-L-Pro-L-Glu-D-Ser-L-Thr and shared the common amino acid sequence Asn-Tyr-Asn with the other iturin members (Peypoux *et al.,* 1981). Bacillomycin D has a monomeric structure, its molecular weight of 1039 calculated for one lipid /I-amino acid and a heptapeptide moiety (Fig.2) was in good agreement with the experimental value, determined by the thermo-osmotic method, of 1060±32 (Peypoux *et al.,* 1984).

Bacillomycin Lc differs from Bacillomycin L by sequence changes from aspartate-1 to asparagine-1 and from glutamine-5 to glutamate-5. Also by the sequence positions of a side chain amide and a carboxylic acid which are at position 5 (L-Glutamine) and at position 1 (L-aspartic acid) respectively in case of bacillomycin L (Eshita *et al.,* 1995). The structure of Bacillomycin F was confirmed as a mixture of homologous peptidolipids, essentially $C_{51}H_{80}N_{12}O_{14}$ and $C_{52}N_{82}N_{12}O_{14}$. The lipid moiety consists of

minor isoCI5, anteisoC15 ß-amino acids and major isoC16, isoC17 and anteisoC17 ß-amino acids (Peypoux *et al.,* 1985).

Biosynthesis of bacillomycins

The non-ribosomal synthesis of peptide antibiotics is widespread among bacteria and fungi (Finking and Marahiel, 2004). Large multienzymes or multimodular proteins termed non-ribosomal peptide synthetases (NRPSs) that are composed of modularly arranged catalytic domains (Fig.3), catalyse all necessary steps in peptide biosynthesis including the selection and ordered condensation of amino acid residues (Stein, 2005; Chen *et al.,* 2009).

Each elongation cycle in non-ribosomal peptide biosynthesis needs the cooperation of three core domains: (i) The adenylation domain (550 amino acid residues) selects its cognate amino acid and generates an enzymatically stabilized amino-acyl adenylate. This mechanism resembles the amino-acylation of tRNA synthetases during ribosomal peptide biosynthesis. (ii) The thiolation or peptidyl carrier domain (80 aa) is equipped with a 4'-phosphopantetheine (PPan) prosthetic group to which the adenylated amino acid substrate is transferred and thioesterified under release of AMP.

Thus, the PPan cofactor acts as thiotemplate and as a swinging arm to transport intermediates between the various catalytic centres. The peptidyl carrier proteins are post-translationally converted from inactive apoforms to their active holoforms by dedicated PPan transferases (Lambalot *et al.,* 1996). (iii) The formation of a new peptide bond is catalysed by condensation domains (450 aa) located between each pair of adenylation and peptidyl carrier domains. The linear assembly line-like arrangement of multiple of such core units (i–iii) ensures the co-ordinated elongation of the peptide product (Stein, 2005; Chen *et al.,* 2009).

In most of the cases the non-ribosomal peptide biosynthesis is terminated by macrocyclization of the peptide product, whereby parts of the molecule distant in the constructed linear peptide chain are covalently linked to one another (Kohli and Walsh, 2003).

Typically, such reactions are catalysed by thioesterase domains at the C terminal end of the NRPS assembly line. The depicted mechanism of peptide biosynthesis has been outlined in the concept of the 'Multiple Carrier Model of Non-ribosomal Peptide Biosynthesis at Modular Multienzymatic Templates' (Stein *et al.,* 1996). Mechanistically, NRPSs utilize multiple Ppan carriers for covalent binding of monomers and growing chains following colinearity rule (Guenzi *et al.,* 1998; Stein, 2005). The system is highly flexible in which naturally rearrangements can be easily achieved within a relatively short period, permitting the random evolution of compounds that provide selective advantages. Striking examples for such flexibility are the systems specifying the biosynthesis of the closely related compounds of the iturin family (Stein, 2005) (Fig.3).

Biosynthetic gene cluster of bacillomycin

The gene clusters devoted to non-ribosomal synthesis of cyclic lipopeptides, surfactin, fengycin and iturin-like antibiotics are widely spread in *B. subtilis* and related strains (Stein, 2005). The first evidence for bacillomycin D enzymatic synthesis through activation of amino acids was provided by Besson and Michel (1992) who purified an enzyme fraction which catalyzed the adenylation of amino acids present in the lipopeptide. Similarly, the mycosubtilin operon is the first operon encoding an iturin lipopeptide that was completely sequenced (Duitman *et al.,* 1999).

Then the modular organizations of other peptide synthetase gene clusters were sequenced like iturin A synthetase operon and compared the adenylation and condensation domains within the iturin group peptides (Tsuge *et al.,* 2001; Moyne *et al.,* 2004).

The operon encoding the bacillomycin D synthetases in *B. subtilis* AU195 was identified, cloned and characterized by Moyne *et al.,* (2004). The Bacillomycin D operon spans 37.8 kb with 4 ORFs: *bamD, bamA, bamB* and *bamC* which share the structural organization of the Mycosubtilin and Iturin A operon (Moyne *et al.,* 2004). In *B. amyloliquefaciens* FZB42, the *bmy* gene cluster is an insertion within its genome and separated by only 25 kb from the fengycin gene cluster. It comprised of four genes (*bmyD, bmyA, bmyB* and *bmyC*) without counterparts in *B. subtilis* 168 (Chen *et al.,* 2009).The ORFs encoding Bmy A (3,982 amino acids), Bmy B (5,633 amino acids), and Bmy C (2,619 amino acids) are organized like their respective counterparts in the iturin A and mycosubtilin operons (Fig.4). They show strong sequence similarity with those components and consist of an ordered arrangement of domains involved in condensation, adenylation, and thiolation (Chen *et al.,* 2009). Seven amino acid-activating modules can be distinguished: A1, located in Bmy A; Bmy B1, Bmy B2, Bmy B3, and Bmy B4, located in Bmy B, and C1 and C2, located in Bmy C. The modules B1, B2, and C1 also contain epimerization domains, directing conversion of amino acids 2, 3, and 6 in a D -configuration. The last domain of this multienzyme system is a thioesterase domain, which is presumably required for release and circularization of the synthesized lipopeptide molecule (Koumoutsi *et al.,* 2004; Chen *et al.,* 2009).

In *B. amyloliquefaciens* GA1, the *itu* operon directing the synthesis of iturin A in *B. subtilis* RB14 (Tsuge *et al.,* 2001) and the bmyL gene cluster in *B. subtilis* A1/3 (Hofemeister *et al.,* 2004) was surprisingly found inserted at exactly the same position as expected bacillomycin D gene cluster from *B. amyloliquefaciens* FZB42. This suggested that an inter-species horizontal transfer of genes could have occurred between *B. subtilis* and *B. amyloliquefaciens* (Arguelles-Arias *et al.,* 2009).

The evidence of main antifungal agent of *B.* amyloliquefaciens FZB42, bacillomycin D that is regulated in multiple layers was reported by Koumoutsi *et al.,* (2007). Expression of the bacillomycin D operon (*bmy*) is dependent on a single σ^A-dependent promoter, P_{bmy} and is favored in its natural host by the small regulatory protein DegQ.

The global regulators DegU and ComA are required for the full transcriptional activation of *bmy*. DegU retains a key role since it binds directly to two sites located upstream of the bacillomycin D promoter. Moreover, both Deg U and a transmembrane protein of unknown function, Ycz E, act on a later level of gene expression, exerting their posttranscriptional effects in a hitherto-unknown manner (Koumoutsi *et al.,* 2007).

Detection of bacillomycin

Stankovic *et al.,* (2012) tested 205 natural isolates of *Bacillus* spp. for the presence of biosynthetic genes of antimicrobial lipopeptides, iturin, surfactin, fengycin and bacillomycin D. The results of the screening showed that the majority of tested strains had more than one biosynthetic operon, since 81% possessed the genes for bacillomyin D production, 54% for surfactin, 38% for iturin and 25% for fengycin production, among which the genes for bacillomycin D were highest in the tested strains of *Bacillus* spp. (Stankovic *et al.,* 2012).

Mode of action of bacillomycins against plant pathogens

The antagonistic effects of the iturin cyclic peptides are the result of their interaction with the cell membrane and formation of pores (Maget-Dana and Peypoux, 1994). The Tyr residue at position 2 in the peptide ring of peptides from the iturin family has a significant role in the mechanism of pore formation in target cells (Harnois *et al.,* 1989, Volpon *et al.,* 1999). The antimicrobial activity of iturins depends predominantly on its capacity to increase membrane permeabilization, which is being attributed to aggregates formed by iturin molecules, iturin–phospholipid complex or iturin–phospholipid–sterol complex (Maget-Dana and Peypoux, 1994). The underlying mechanism is based on osmotic perturbation owing to the formation of ion-conducting pores and not membrane disruption or solubilization as caused by surfactins (Aranda *et al.,* 2005).

The detailed study of antimicrobial activity of *Bacillus subtilis* B-38, isolated from soil against human pathogenic spp. of *Candida albicans* was done by Tabbene *et al.,* (2011). They revealed the presence of *bamC* gene producing bacillomycin D and 3 anti-candida compounds were purified from culture supernatants of the *B. subtilis* which were identified as analogues of bacillomycin D of 14, 15 and16 carbon fatty acid long chains by using MALDI-TOF-MS (Tabbene *et al.,* 2011). These findings suggest that acyl chain length of bacillomycin D like lipopeptides plays a major role in hemolytic and antifungal activities.

Bacillomycin L belonging to the iturin family was originally isolated from the culture broth of *B. amyloliquefaciens* K103 (Zhang *et al.,* 2013). To understand the actual interaction of iturin family antibiotics with fungal membranes they have studied the antifungal activity of bacillomycin L which involves specific interaction with the intact fungal hyphae of *R. solani* Kühn and investigated in detail by means of various fluorescent techniques, electron microscopy and gel retardation experiment assay. They concluded that the inhibitory activity of bacillomycin L against *R. solani* Kühn not only solely involve membrane permeabilization, but also link to the interaction with the intracellular targets, such as cell 's DNA (Zhang *et al.,* 2013). The antifungal activity of bacillomycin D extracted from *B. subtilis* strain fmbJ against *Aspergillus flavus* was studied which caused severe injury to both cell wall and cell membrane of fungal spores and hypha (Gong *et al.,* 2014).

Biological control of plant diseases by using *Bacillus* spp. with AMP (bacillomycins) genes

There has been tremendous research in identification of worthy and notable biocontrol agents, especially *Bacillus* strains with NRPSs lipopeptide genes which would offer better and more efficient disease control. Ramarathnam *et al.,* (2007) identified for the presence of *bamC* gene of bacillomycin D synthetase biosynthetic operon in *Bacillus cereus* DFE4, *Bacillus amyloliquefaciens* strains DFE16 and BS6, and *Bacillus subtilis* 49. Both Fengycin and Bacillomycin D were detected in the culture extract of strain BS49, characterized through MALDI-TOF-MS, and their antifungal activities were demonstrated against *F. graminearum* and *Sclerotinia sclerotiorum,* common fungal pathogens of Canola (Ramarathnam *et al.,* 2007). Likewise, the antagonism of *B. subtilis* strains towards *Podosphaera fusca* causing cucurbit downy mildew was studied by identifying 3 lipopeptide antibiotics, surfactin, fengycin and iturin A or bacillomycin in butanolic extracts from cell culture filtrates (Romero *et al.,* 2007).

Fig.1 Primary structures of bacillomycin lipopeptides

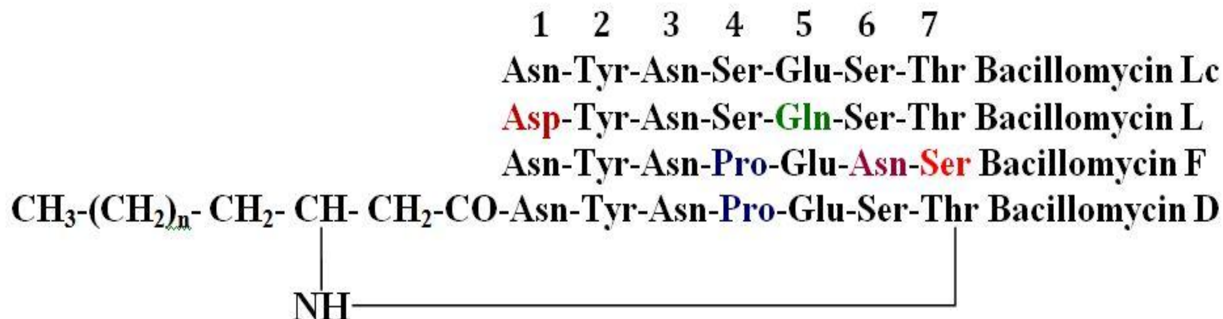

Fig.2 Structure of bacillomycin D (IUPAC name: 3-amino-l2-methyl-tri-decanoic acid or 3-amino 12-methyl tetradecanoic acid)

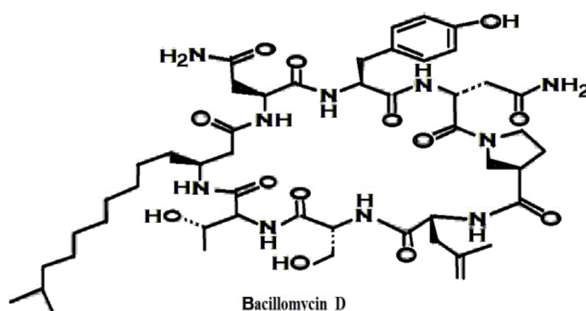

Fig.3 Non-ribosomally synthesized peptide antibiotic bacillomycin D in *B. subtilis* AU195. In each line, the genetic organization of the NRPSs (boxed) and schematic representations of produced peptide antibiotics and their possible isoforms are given. Amino acid residues, usually in L-configuration, are shown in the single-letter code, and residues in D-configuration are underlined; the fatty acid moieties are hatched and the number of their carbon atoms are indexed (Ci). Each modularly arranged elongation unit contains a domain for adenylation of the amino acid substrate, a peptidyl carrier protein (PCP) and a condensation domain where the formation of a new peptide bond occurs. In the case of amino acids in D-configuration, the NRPSs contain an additional epimerase domain. Numbers correspond either to the size of the gene clusters (in kb) or to the derived molecular mass of the NRPSs (in kDa). The iturin lipopeptide family share a ß-amino fatty acid as integral constituent, positions 1–3 of the peptide moiety (L-Asx-D-Tyr-D-Asx) and an additional D-amino acid at position 6 (Stein, 2005)

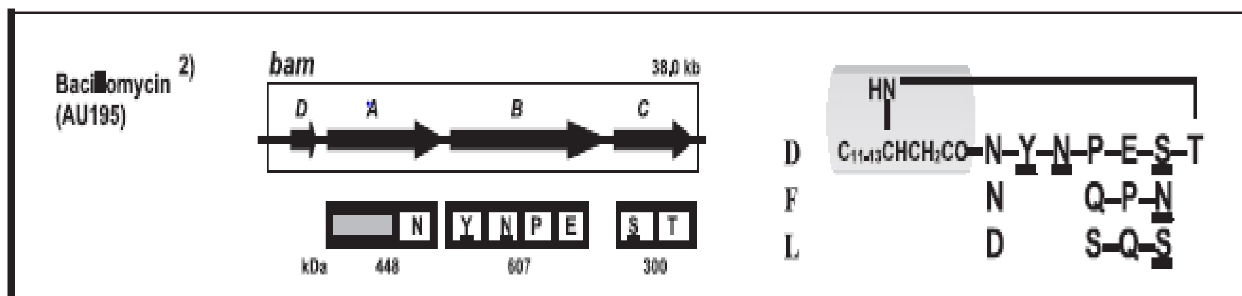

Fig.4 Gene cluster of bacillomycin D (37kb) in FZB42 (Chen *et al.,* 2009)

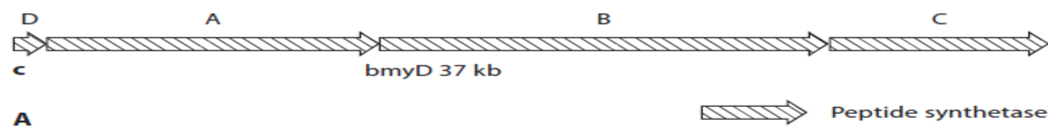

Table.1 Primers for the detection of bacillomycin D biosynthetic genes (Chung *et al.,* 2008)

Antibiotic	Gene(s)	Primers and sequences	PCR product size expected/ detected	Accession number/ Reference
Bacillomycin D	*bam D*	ITUD-F1 5′-TTGAAYGTCAGYGC SCCTTT	482 bp/Yes	AB050629, ATTCAU195
		ITUD-R1 5′-TGCGMAAATAATGG SGTCG		L42526, AF499447
	bam C	BAMC-F1 5′-AGTAAATGAACGCG CCAATC	957 bp/No	AY137375
		BAMC-R1 5′-CCCTCTCCTGCCAC ATAGAG		AY137375

The purified lipopeptide fractions strongly inhibited the conidia germination of *P. fusca* which were supported by site-directed mutagenesis analysis, targeted to suppress the biosynthesis of different lipopeptides and concluded the major role of iturin and fengycin families in the antagonism of *B. subtilis* towards *P. fusca* (Romero *et al.,* 2007).

The *B. subtilis* ME 488 isolate suppressed the growth of *F. o.* f.sp. *cucumerinum* and *Phytophthora capsici* on pepper in pot assays. PCR study confirmed the presence of 11 antibiotics in the *Bacillus* isolates in which bacillomycin D was also present (Chung *et al.,* 2008). The bacterium *B. subtilis* H215 isolated from honey showed high antifungal activity against *Byssochlamys fulva* H25, a spoilage mould of juices and beverages (Lee *et al.,* 2008). The purified AMP was found with 5 active fractions which were lyophilized and subjected to mass spectrometry, wherein the five peaks were determined to be identical to bacillomycin F, varying in the length of the fatty acid chain moiety from C14 to C16. The AMP was found to be stable over a wide pH range and upto 100ºC temperature which was first report of honey microflora producing bacillomycin F or any antifungal compound (Lee *et al.,* 2008).

Biological activity of *Bacillus amyloliquefaciens* FZB42 against *Fusarium oxysporium* was found due to the presence of bacillomycin D mainly (Koumoutsi *et al.,* 2004). The complete genome sequence of this strain FZB42 was given by Chen *et al.,* (2007). The *bmy* gene cluster was found to be an insertion within the FZB42 genome and separated by only 25 kb from fengycin gene cluster and they described the synergism of both bacillomycin D and fengycin by producing double mutants for respected

lipopeptide gene via gene replacement strategy (Koumoutsi *et al.,* 2004). Similar results were produced in FZB42 strain for its biocontrol activity and respective mutants for all the lipopeptide genes present were assayed in direct growth tests and by bioautography (Chen *et al.,* 2009).

Bacillus amyloliquefaciens FZB42 inhibited growth of plant pathogenic fungi as *Fusarium* spp. including *F. oxysporum*, *Gaeumannomyces graminis*, *Rhizoctonia solani*, *Alternaria alternata* and *Pythium aphanidermatum* whilst mutant strains deficient in bacillomycin D production were severely impaired in their antifungal activity suggesting that bacillomycin D contributes significantly to its antifungal activity (Chen *et al.,* 2009).

Interestingly the antagonism of bacillomycins produced by *Bacillus* isolates against the lipopeptides of pathogens is also being studied. The activity of bacillomycin D (n-C14) and bacillomycin D (iso-C15) isolated from *B. vallismortis* ZZ185 against the activity of the lipopeptides of fungal pathogens, *F. graminearum, A. alternata, R. solani, C. parasitica* and *P. capsci* was elaborated by Zhao *et al.,* (2010).

Moreover, antagonistic endophytic bacterial species are taken for biological control from the hosts in order to control the particular diseases which are having huge antimicrobial activity and plant growth promoting factors rather than the one isolated from the rhizosphere or phyllosphere of the concerned host plants. An endophytic bacterium, *B. amyloliquefaciens* CGMCC 5569 was isolated from medicinal plant *Ginkgo biloba* showed growth inhibition activity (> 65%) against sapstain fungi *Lasiodiplodia rubropurpurea, L. crassispora* and *L. theobromae* which is a serious problem of timber, paper and pulp producers based on petriplate study. The antifungal compounds were obtained as a series of lipopeptides, fengycin, surfactin and bacillomycin (Yuan *et al.,* 2012).

The endophytic *Bacillus* spp. were isolated from seeds of several varieties of maize, Indian popcorn and yellow dent corn varieties which were tested for their antifungal activity against *Fusarium moniliforme* (Gond *et al.,* 2015). The presence of antifungal lipopeptides genes of iturin A, fengycin and bacillomycin was detected by MALDI-TOF-MS. Also they tested the induction of defense gene expression in the host after treating them with *B. subtilis* (SG-JW-03), wherein the treated roots showed the induction of PR-genes, PR-1 and PR-4 (Gond *et al.,* 2015).

The antimicrobial peptides (AMPs) have wider diversity in their applications commercially, but with the limitation of high production costs in huge quantities.

To reduce this high production costs researchers have utilized low cost raw substrates to produce AMPs or lipopeptides such as rice straw (Zhu *et al.,* 2013), potato (Fox *et al.,* 2000), orange peels (Ghribi *et al.,* 2011) and waste frying oils (Li *et al.,* 2016).

The primary antifungal substances, bacillomycin L, fengycin and surfactin were isolated from cell suspension of *Bacillus amyloliquefaciens* SYBC H47 isolated from raw honey against *Botryosphaeria dothidea* under laboratory and field conditions, among which bacillomycin L showed the best inhibitory effect against conidial germination of *B. dothidea*. In order to raise the lipopeptide yield levels waste frying peanut oil and soy oil as the sole carbon source was utilized and in field trial, the decrease in infected gummosis rate (IGR) and disease severity index (DSI) through cell free suspension treatments of *B. amyloliquefaciens* SYBC H47 was observed (Li *et al.,* 2016).

The antagonistic bacteria *Bacillus subtilis* PSB5 (KJ817861), *B. amyloliquefaciens* PSB6 (KJ817862) and *B. tequilensis* PSB8 (KJ817864) were found to be very effective against *F. o.* f. sp. *gerberae* (FOG) (KJ570974) both under *in-vitro* and protected cultivation conditions with plant growth promoting activities as it was comprising the AMP producing genes as *Itu C, Itu D, Bam C, Fen D, Srf A, SfP* (Suneeta *et. al.,* 2016a). Similarly the same strains of *Bacillus* (PSB6 and PSB8) carrying the lipopeptide biosynthetic genes were found to be highly effective under laboratory and field conditions against the polyphagous pathogen *Sclerotium rolfsii* (first report in *Gerbera jamesonii* in Tamil Nadu, India) causing collar rot disease in *Gerbera* (Suneeta *et al.,* 2016b).

A strain of *Bacillus subtilis* namely Czk1 was isolated from the aerial roots of rubber trees which exhibited the strongest antagonistic activity against *Ganoderma pseudoferreum*, *phellinus noxius*, *Helicobasidium compactum, Rigidoporus lignosus, Sphaerostilbe repens* and *Colletotrichum gleosporiodes* (He *et al.,* 2017). The crude antibiotic extract was purified, cloned the lipopeptide genes and analysed by MALDI-TOF-MS as surfactin, iturin, fengycin and bacillomycin antibiotics suggesting the strain Czk1 as potential disease biocontrol and is the first report of *B. subtilis* strain from *Hevea brasiliensis* co-producing these many variants of lipopeptides (He *et al.,* 2017).

The volatile and agar diffusible antifungal metabolites produced by *Bacillus velezensis* G341 isolated from 4-year old roots of Korean ginseng with strong antifungal activity against various phytopathogenic fungi due to the production of 2 antifungal compounds, bacillomycin L and fengycin A and volatile compounds emitted as DMSO, 1-butanol and acetoin identified from GC-MS (Lim *et al.,* 2017).

An outlook

Among the antimicrobial compounds, cyclic lipopeptides (LPs) of the iturin, surfactin and fengycin families have well recognized wider uses in biomedicine and biotechnological applications because of their surfactant, antimicrobial and drug transportation properties. In this review, we have tried to give a picture about bacillomycins in a frame of its entire molecular studies with a great commercialization potential. So far there is few research and patents on individual bacillomycin antibiotic commercial production from its promising *Bacillus* strains, though its importance is incredible in antifungal activity against plant diseases. Inulin was proved to be the efficient enhancer of bacillomycin D production from *B. subtilis* strain fmbJ which promoted the bacillomycin D biosynthetase gene (Qian *et al.,* 2015). Also they tested L-glutamine singly and in combination with inulin which significantly raised the bacillomycin D production to 1.93 g/L (Qian *et al.,* 2017).

Although, there are limitations with the iturin lipopeptides in case of human drug research area, there have been further studies designed to improve the potential and to reduce the side-effects of bacillomycins successfully. Bacillomycins are specially known for their anti-yeast feature (Tabbene *et al.,* 2011) against dangerous human pathogen *Candida* spp. but with a hemolytic activity as described above. So the toxic effects, drug resistance and hemolysis activity have been tried to reduce by combining two or more synergistic antimycotic drugs like the synergism of polyene drug amphotericin B and bacillomycin D have been described by Tabbene *et al.,* (2015) against *Candida* spp.

Moreover, there is tremendous research on *Bacillus* strains producing lipopeptides, their comparative, evolutionary and functional

genomics, site-directed mutagenesis and strain construction studies including marker removal was successfully done like in *B. amyloliquefaciens* FZB42 (Wu *et. al.,* 2015) which is almost done as a genetically-engineered (GE) strain against broader range of plant pathogenic fungi. But, reasonable lacunae are present in understanding many molecular mechanisms of the BCAs in plant spheres to further pave a way in achieving commercialization of a lipopeptide antibiotic or a GE strain of BCAs successfully.

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How to cite this article:

Suneeta, P., K. Eraivan Arutkani Aiyanathan and Nakkeeran, S. 2018. Bacillomycins – The Effective Molecules in Plant Disease Management. *Int.J.Curr.Microbiol.App.Sci.* 7(02): 823-835. doi: https://doi.org/10.20546/ijcmas.2018.702.104