

Review Article

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**Influence of Biomolecules of *Bacillus* spp.
against Phytopathogens: A Review**

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Literature available on the association of biomolecules with *Bacillus* spp. and their influence on phytopathogens is very interesting and it needs indepth study to understand the mechanism of *Bacillus* in the management of phytopathogens. The available literature on this aspect revealed the association of several biomolecules with *Bacillus* and their role in affecting nematodes. The information on this line is imperative to understand the molecular mechanism of biomolecules over nematodes. Therefore information on this line is pooled and presented as mini review to programme future line of work.

Introduction

Biomolecules Associated with *Bacillus* spp.

The *Bacillus* spp. capable of producing a wide variety of secondary metabolites are diverse in their structure and function. The capacity of production of antimicrobial metabolites by *Bacillus* spp. determines their ability to control plant diseases (Silo-suh *et al.*, 1994). Surfactin and iturin A are the most common lipopeptide antibiotics produced by *Bacillus* spp. Antibiotics from iturin family is showing strong antifungal and hemolytic activities but with limited

antibacterial activity. Surfactin, an acidic cyclic lipopeptide produced by *B. subtilis* is considered as a biosurfactant by Maget-Dana and Ptak (1990) is having greater value in the application of chemical pesticides. The *Bacillus* spp. possessing iturin known for broad antifungal spectrum serves as a potential biocontrol agent in the management of wide array of plant diseases according to Maget-Dana and Peypoux (1994).

Hence, the detection of the bacterial isolates possessing both iturin and surfactin is highly imperative to consider them as desirable bioagent in plant protection. The antibiotic

biosynthetic genes iturin and surfactin detected through TLC with *B. weihenstephanensis* (TSB4), *B. subtilis* (TSB5) and *B. licheniformis* (TSB4) made them as effective isolates to check *M. incognita* and *F. oxysporum* f.sp. *lycopersici* individually as well as concomitantly (Tamalika Sarangi, 2014).

Hence, the detection of both iturin and surfactin through TLC in the isolates of *B. weihenstephanensis* (TSB4), *B. subtilis* (TSB5) and *B. licheniformis* (TSB4) as first hand information led to the in-depth study on chemical characterization of active molecules for better understanding of their mechanism in the suppression of root knot nematode, fungus *Fusarium* and nematode fungal disease complex in tomato under controlled and glasshouse conditions.

Although in many instances the TLC was performed for the detection of antibiotic biosynthetic genes present in the *Bacillus* spp. proved to be effective against fungus viz. *Curvularia* spp., *Alternaria* spp., *Aspergillus niger* and *Candida albicans*, bacteria (*Artemia* spp.) and algae (*Klebsilla* spp.) not much work have been carried out with the endophytic *Bacillus* to prove as highly nematocidal (Ajilani and Hasnain, 2006 and Gupta *et al.*, 2014).

The GC-MS analysis was performed for the chemical characterization of biomolecules of the most effective endophyte of *B. weihenstephanensis* (TSB4) which proved to be effective against fungal and nematode diseases (Tamalika Sarangi, 2014).

With increased importance of phytopathogens causing plant disease, the search for a new novel bioagent has gained urgency. The production of bioactive compounds by *Bacillus* spp. is well established by Korzybski *et al.*, (1978), Naruse *et al.*, (1990), Munimbazi and

Bullerman, (1998). In this juncture Pabel *et al.* (2003) opined that these classes of bioactive compounds are acting as antifungal peptides, antifungal lipopeptides and antimicrobial polypeptides.

With regard to *Bacillus* spp., Pumilacidin as cyclic acylheptapeptide composed of a beta-hydroxy fatty acid is reported from *B. pumilus* (Naruse *et al.*, 1990). This lipopeptide has antimicrobial (Pabel *et al.*, 2003), antiviral and antiulcer activity (Naruse *et al.*, 1990).

Effect on Phytonematodes and other pathogens

Against Root Knot Nematode

Three families of *Bacillus* lipopeptides viz. surfactins, iturins and fengycins are the most studied biomolecules for their antagonistic activity against a wide range of phytopathogens including bacteria, fungi and nematodes. In this study, the strain Bbv 57 of *B. subtilis* isolated from banana and compared as check showed remarkable antinematic property. The genomic DNA of the *Bacillus* strain was isolated and amplified by PCR to identify antibiotic genes. The biosynthetic gene specific primers amplified a 440 bp fragment of *surfactin* gene from *B. subtilis* strains of BsN 3, Bs 5 and Bbv 57 and a 648 bp fragment of iturin gene from the strains of Bs 5 and Bbv 57. The *B. subtilis* strain Bs 5 exhibited the highest surfactin and iturin activity *in vitro* was found to be capable of suppressing egg hatching and mortality of second stage juveniles of *M. incognita* (Kavita *et al.*, 2012).

The Purl gene of *B. subtilis* strains OKB105 and 69 and *B. amyloliquefaciens* strains of FZB42 and B3 were found to be nematocidal against root knot nematode according to the report of Xia *et al.* (2011).

Against other nematodes

The Purl gene of specific strains of *B. subtilis* (OKB105 and 69) and *B.amyloliquefaciens* (FZB42 and B3) reported to exhibit nematicidal property against *M.javanica* were also effective against *Aphelenchoides besseyi*, *Ditylenchus destructor*, *Bursaphelenchus xylophilus* (Xia *et al.*, 2011).

Against Fungal Pathogens

Genetic diversity of *Bacillus* strains isolated from rhizospheric soil as endophytes was studied for their potentiality to control bacterial wilt of tomato. In this study with 250 isolates of *Bacillus* spp. collected from different agro-climatic regions of India, 47 strains showed antagonistic ability against *R. solanacearum*. The growth of *R.solanacearum* was inhibited by the strain DTBS-5 at highest level under glasshouse conditions. However the tomato plants treated with the strain of JTBS-9 had maximum fresh weight. Further, out of 47 strains 11 strains of *Bacillus* spp. were detected as iturin antibiotic producing strains using iturin gene based marker. Genetic variability was observed in *Bacillus* spp. which was made with five clusters at 50 per cent similarity coefficient. However, iturin producing and iturin nonproducing strains as well as rhizospheric and endophytic *Bacillus* spp. could not be distinguished using 16S rRNA sequence analysis and genetic finger printing as stated by Singh *et al.*(2013).

F.graminearum causes *Fusarium* head blight, a devastating disease that leads to extensive yield and quality loss of wheat and barley. The bacteria isolated from wheat kernels and plant anthers were screened for their antagonistic activity against *F.graminearum*. Based on *in vitro*

effectiveness the strain SG6 was selected for characterization and it was identified as *B.subtilis*. The bacterium strain exhibited a high antifungal effect on the mycelium growth, sporulation production of *F.graminearum* with the inhibition rate of 87.9, 95.6 and 100 per cent respectively. The antifungal activity of the strain SG6 could be attributed to the coproduction of chitinase, fengycins and surfactins (Zhao *et al.*, 2012).

An antifungal lipopeptide fengycin producing strain isolated from farm land soil sample was identified as *B. thuringiensis* strain SM1 using 16S rDNA analysis. The strain is reported to possess antibacterial property by Roy *et al.* (2013).

A novel phospholipid antibiotic *viz.* bacilysoicin accumulated within the cells of *B. subtilis* strain 168 was subjected to determine the structure of active compounds using nuclear magnetic resonance and mass spectrometry analyses. The structure of bacilysoicin elucidated as 1- (12-methyltetradecanoyl)-3-phosphoglycerol is responsible for the possession of antimicrobial activity especially against certain fungi (Tamehiro *et al.*, 2002).

The genome of *B.amyloliquefaciens* Q-426 effective against plant pathogens was tested for its potential use against a variety of plant pathogens. The screening of genes involved in the biosynthesis of antifungal agents revealed that the *fen* and *bmy* gene clusters are present in the Q-426 genome. Lipopeptides such as bacillomycin D, fengycin A and fengycin B purified were found to inhibit spore germination of *F.oxysporum* (Zhao *et al.*, 2013).

The endospore forming rhizobacteria *B.subtilis* producing several antibiotics *viz.* subtilosin, bacitracin, difficidin, fengycin,

mersacidin, bacilysoicin and iturin were proved to be effective against both gram positive and gram negative bacteria. Environmental conditions induce microorganisms to produce varied kinds of antimicrobials which have applications in chemotherapy. The purified phospholipid antibiotic of acidophilic *B.subtilis* strains were tested against *Escherichia coli*, *Staphalococcus aureus*, *Pseudomonas aeruginosa* and *Candida parapsilosis*. The antibiotics of *B. subtilis* showed broad spectrum activity over the above mentioned pathogens (Mora *et al.*, 2011).

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