

# Biological control of fungal phytopathogens: A comprehensive review based on *Bacillus* species

## Abstract

Worldwide, emergence of new resistant fungal phytopathogens and use of chemical fertilizers, pesticides, fungicides for the protection of crops lead to serious environmental issues. Due to these problems, the need to explore alternative management approaches that are not only successful but also less harmful to the environment is of paramount importance. *Bacillus species* are a good candidate for use as a biocontrol agent against these fungal phytopathogens because they produce various types of antimicrobial substances and their environmentally friendly behavior. This article provides basic knowledge of *Bacillus* spp. biocontrol agents, tools for identification of their biocontrol agents and techniques for screening of *Bacillus* spp. against fungal phytopathogens based on current findings. Specifically, if thoroughly explored, *Bacillus* will remove the existing commonly used control agents such as fungicides, harmful cultural activities of health and control phytopathogens in the ecosystem.

**Keywords:** bacillus, emergence, phytopathogen, biocontrol

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Muddasir Khan,<sup>1</sup> Muhammad Salman,<sup>1</sup> Sohail Ahmad Jan,<sup>2</sup> Zabta Khan Shinwari<sup>3,4</sup>

<sup>1</sup>Department of Health and Biological Sciences, Abasyn University Peshawar, Pakistan

<sup>2</sup>Department of Bioinformatics and Biosciences, Capital University of Science and Technology, Pakistan

<sup>3</sup>Department of Plant Sciences, Quaid-i-Azam University, Pakistan

<sup>4</sup>Pakistan Academy of Sciences, Pakistan

**Correspondence:** Muddasir Khan, Department of Health and Biological Sciences, Abasyn University Peshawar, Khyber Pakhtunkhwa, Pakistan, Tel 0092-3025678947, ORCID 0000-0001-6545-7616, Email muddasir.khan0302@gmail.com

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## Introduction

Globally, biotic stresses such as pests and pathogens are main factors of threat for the production of crops and a recently study reported that crop loss about 8-40% worldwide is due to fungal pathogens.<sup>1</sup> Among these plant diseases, the most important economic pathogen is *Fusarium* species, which cause a wide range of diseases including wilts, head blights, fruit rots, and root rots. The population of disease caused by *Fusarium graminearum* was reported in the Netherlands, western Canada, Great Britain, Germany, northwest Europe and North America.<sup>2</sup> Globally, a major crops loss is due to the disease cause by *Rhizoctonia solani* which is a most important fungal phytopathogen belongs to genus *Rhizoctonia*. This fungus is the prominent causative agent of soil-borne diseases including root rots, wilts and seedling disease.<sup>3</sup> Additionally, grain quality reduction fungus named *F. graminearum* produce mycotoxin such as deoxynivalenol (DON), trichothecenesivalenol (NIV), its derivatives 3-and 15- acetyldeoxynivalenol (3-ADON, 15-ADON) which cause diseases in plants, immunosuppression and neurological disorder in humans and animals.<sup>4</sup> Based on scientific and economic importance, *F. graminearum* is the fourth important fungal phytopathogen. It affects cereal crops and a major source of contamination in food grain. The associated symptoms of this fungal infection are premature bleaching due to which yield and quality of the grain is reduce.<sup>1</sup> *R. solani* symptoms on varied hosts include crown rot, root rot, seed rot, hypocotyl rot, limb rot, stem rot, stem canker, pod rot, pre- & post-emergence damping off, black scurf and seedling blight.<sup>3</sup> To monitor these plants diseases, therefore, exploring alternative management approaches which are not only successful but also less harmful to the environment.

From the last three decades, various bio-control strategies have been developed in the form of chemical fertilizers, pesticides, and fungicides against pests and fungal phytopathogens for the protection of crops. The use of these chemicals and agents lead to serious environmental issues. The plant growth promoting rhizobacteria (PGPR) offer an attraction to replace these chemicals and fertilizers

by the biological agents due to their wide range availability, biocontrol agent's production and environmental friendly behavior.<sup>5,6</sup> The PGPR prevents the growth of the phytopathogens through their antagonistic activity. They suppress the growth of the pathogen and produce antibiotics, organic volatile compounds, hydrolytic enzymes, extracellular antifungal metabolites, cellulolytic enzymes such as proteases, celluloses, chitinases and cyanides. The PGPR also reduces the growth of the phytopathogens through nutrient competition.<sup>7</sup> Among the genera of bacteria, the reported most common rhizobacteria include; *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Agrobacterium*, *Rhizobia*, *Alcaligenes*, *Azotobacter*, *Mycobacterium*, *Flavobacter*, *Cellulomonas*, and *Micrococcus*.<sup>8</sup> The PGPR is a good candidate to use as bio-control agents against phytopathogens because of their environmentally friendly behavior. This prospective article provides basic literature knowledge about certain *Bacillus* species as bio-control agents against fungal phytopathogens like *Fusarium graminearum* and *Rhizoctonia solani*.

## *Bacillus* species against phytopathogens

In the field of biopesticides, the *Bacillus* species and other PGPR play an important role because they produce a variety of antimicrobial agents including lipopeptides, antibiotics, and enzymes which promote the growth of plants and inhibit the pathogenic microorganisms.<sup>9</sup> The *Bacillus* species are found mostly in soil and produce metabolites, which have antibiotic properties and are capable to reduce or suppress the growth of another microorganism.<sup>10</sup> *Bacillus* species also produce some volatile agents to promote the plant defense mechanism. Through the biocontrol activity of *Bacillus* species, mostly bacterial and fungal phytopathogens have been controlled and effective against the protection of plants from diseases. The antimicrobial substances produced by *Bacillus* species include; subtilin, bacilysin, mycobacillin, bacillomycin, mycosubtilin, iturin, fengycin, and surfactin which has both antifungal and antibacterial activity.<sup>1</sup> *Bacillus* species are a good candidate for use as a biocontrol agent because they produce various types of antimicrobial substances and their environmentally friendly behavior.

## Biocontrol agents of *Bacillus* species

*Bacillus* species produce various types of biocontrol agents, which are differentiated by their chemical structure,<sup>11</sup> among these the fengycins, iturins and surfactins are reported in various studies conducted by researchers.<sup>1</sup>

**Fengycin:** Fengycin is a cyclic lipodecapeptide composed of  $\beta$ -hydroxy fatty acid with 16-19 carbon atoms in their side chain.<sup>11</sup> According to the previously reported study, the fengycin is mostly produced by *Bacillus amyloliquefaciens* and *Bacillus subtilis*.<sup>12,13</sup> Fengycin inhibits the growth of filamentous fungi. They act on the biological membranes and completely disrupt the membranes in different concentration, also inhibits the fungal enzymes such as aromatase and phospholipase A2.<sup>14</sup> Fengycin promotes the growth of the plant<sup>15</sup> and effective against various type of fungus including *Magnaporthe grisea*, *Plasmodiophora brassicae*, *Botryosphaeria dothidea*, *C. gloeosporioides*, *F. verticillioides*, *F. solani*, *F. solani* f. sp. *radicicola*, *F. oxysporum*, *F. oxysporum* f. sp. *spinaciae*, *F. verticillioides* and *F. graminearum*.<sup>16-20</sup> Fengycins inhibit the growth of *F. graminearum* by disrupting their cell membrane permeability; the inhibition depends upon the concentration of fengycin.<sup>21</sup>

**Iturin:** Iturin is an amphiphilic compounds having C13-to-C17  $\beta$ -amino fatty acid chain connected to heptapeptide backbone and produce ion-conducting pores inside the fungal cell membranes due to their high fungitoxic characteristics.<sup>22</sup> Iturin mostly produce by *Bacillus subtilis* and *Bacillus amyloliquefaciens* strains according to the previously reported studies.<sup>23-26</sup> Iturin exhibit the antifungal activity against a number of fungal phytopathogens including *F. graminearum*, *F. oxysporum*, *R. solani*, *Penicillium*, *Monilinia*, *Botrytis* and *Colletotrichum*.<sup>26,27-29</sup>

**Surfactin:** Surfactin are lipopeptides composed of  $\beta$ -hydroxyhepta cyclic depsipeptides with potential combinations of alanine, valine, leucine or isoleucine amino acids at positions 2, 4 and 7 in the cyclic depsipeptide moiety and variations of C13 to C16 in the chains of  $\beta$ -hydroxy fatty acids.<sup>30</sup> Surfactin exhibits naturally antifungal activity and produce by *Bacillus spp.* according to the reported studies.<sup>13,31</sup> Surfactin inhibits the growth of various fungal phytopathogens includes *Colletotrichum gloeosporioides*, *F. verticillioides*, *F. graminearum*, *F. oxysporum*, *F. moniliforme* and *F. verticillioides*.<sup>20, 32-34</sup>

## Techniques used for the identification of *Bacillus spp.* antifungal compounds

*Bacillus spp.* genomic research has shown that these bacteria contain genes that code for biological control-associated metabolites. Surfactin, fengycin, and iturin culture experiments are regularly conducted and have shown a myriad of antimicrobial substances capable of working against plant pathogenic fungi. To classify compounds that have antagonistic activity, these experiments use chromatography techniques and mass spectrometry. According to the current research on inhibition of *F. graminearum* and biosynthesis of their mycotoxin due to fengycin produce by *Bacillus amyloliquefaciens*. The Fengycin was purified and identified from the crude extract of *Bacillus amyloliquefaciens* by the HPLC techniques. Fengycin substantially impeded *F. graminearum* growth and also decreased the development of their mycotoxins. The authors inferred that *B. amyloliquefaciens* produce fengycin which potentially acts as an agent of biocontrol against *F. graminearum* and mycotoxins produced by this fungus can also be inhibited.<sup>12</sup> Another study reported that the *B. amyloliquefaciens* successfully inhibit or control in vivo and

in vitro environment the growth of *F. graminearum*. The antifungal compound was purified and identified as cyclo (L-Pro-D-Tyr) by spectroscopy. The first time inhibition analysis of cyclo (L-Pro-D-Tyr) against *F. graminearum* colonies was conformed. In vivo experiment by the use of cyclo(L-Pro-D-Tyr) the yield of the crops become 27% higher. The authors inferred that *B. amyloliquefaciens* potentially act as an agent of biocontrol against *F. graminearum* as well as activities to promote plant growth of wheat.<sup>35</sup>

## *Bacillus spp.* screening techniques against fungal phytopathogens

Traditionally, the effect of an organism or a material against the growth of a target organism is analyzed using bioassays. In a bioassay, in the presence of the antagonist, the organism is grown and its development tracked over time as opposed to that of experimental control. A region of growth inhibition around the inhibited microbe is characteristically shaped. To determine the impact of *Bacillus spp.* numerous bioassays have been performed against phytopathogens of fungi. The previously conducted analysis, which explicitly shows an antagonism between *Bacillus spp.* and fungal phytopathogens is noteworthy.<sup>36</sup> According to another notable study, in which the biocontrol potential of rhizobacteria against the plant disease determined. The obtained result shows that *Bacillus spp.* was capable to reduce 67.34% of fungal colonies and also has the potential to improve the growth of the plant.<sup>37</sup> Recently reported study on *B. subtilis* biocontrol against *Rhizoctonia solani* in pepper. The observations indicate that *B. Subtilis* was not only able to enable the mediated systemic resistance of pepper seedlings to induced wilt disease. *R. Solani* signaling pathway dependent on jasmonic acid but also produces antifungal compounds to prevent or even destroy mycelium formation. The results of this analysis offer innovative guidelines for the production of plant safety.<sup>38</sup>

## Conclusion

The main strategies for the protection of crops from diseases are crop rotation, chemical pesticides, and chemical fungicides. Recently, due to the resistance of pathogens to fungicides and environmental issues, the biocontrol pattern of microbes through their antagonistic activity has become most important. This method is safe, environment friendly, and sustainable for the reduction of phytopathogens. Also at traceable speeds, high-resolution chromatography and mass spectrometry techniques can allow the discovery of new antagonistic molecules feasible. Specifically, if thoroughly explored, *Bacillus* will remove the existing commonly used control agents such as fungicides, harmful cultural activities of health and control phytopathogens in the ecosystem.

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## Conflicts of interest

The authors declare that there is no conflict of interest.

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