# Genomic analyses reveal that biocontrol of wheat blast by *Bacillus* spp. may be linked with production of antimicrobial compounds and induced systemic resistance in host plants

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## Abstract

The genomes of three potential biocontrol bacterial isolates, *Bacillus amyloliquefaciens* (BTLK6A), *B. subtilis* (BTS-3), and *B. amyloliquefaciens* (BTS-4) were analysed to gain insights into the underlying molecular basis of their inhibitory activities against the wheat blast fungus *Magnaporthe oryzae Triticum* (MoT) pathotype. Our genomic analyses revealed that biocontrol mechanisms of wheat blast by these probiotic Bacillus species may be linked with production and secretion of antimicrobial compounds and induced systemic resistant to host plants. Our results suggest that probiotic bacteria protect wheat seedlings from artificially inoculated blast fungus through an array of antibiotics and lytic enzymes. Further studies are needed to confirm specific roles of the predicted genes by mutants etc. and isolate and characterize the major antimicrobial compounds from these wheat blast biocontrol bacteria.

## Introduction

Wheat (*Triticum aestivum*) is the second most important cereal crop next to rice in Bangladesh. It plays an important role in attaining food and nutritional security of the increasing population and changing food habit of this highly populated country. Wheat blast has recently emerged as a serious threat to food and nutritional security of Bangladesh, India and other South Asian countries since its first occurrence in Bangladesh in 2016. In February 2016, wheat blast was first spotted in 8 districts of Bangladesh and devastated more thank 15,000 hectares of wheat with yield losses up to 100%.[1)] It is caused by a filamentous fungus *Magnaporthe oryzae Triticum* (MoT) pathotype which is difficult to control by chemical fungicides are unreliable. Genetic sources for breeding resistant varieties are also limited. Novel approaches are needed to tackle this worrisome wheat killer. To develop an effective biological control agent, the aim of this research was to discover plant probiotic bacteria that suppress wheat blast fungus both

in vitro and in vivo. Screening 650 plant probiotic bacteria isolated from various cultivated crops in Bangladesh, we discovered three isolates (BTS-3, BTS-4 and BTLK6A) of *Bacillus* spp. that significantly suppress MoT both in vitro and in vivo.[2)] To better understand the underlying molecular mechanisms of wheat blast control by these native plants associated bacteria, we sequenced the genomes of these bacteria using a whole genome shotgun approach.[3)] This study describes analyses of the genomes of these plant probiotic bacteria to shed light on mechanisms of their inhibitory effects toward MoT. Our results reveal that biocontrol of wheat blast by these three strains of *Bacillus* spp. may be linked with their synthesis and exudation of diverse antibiotics and lytic enzymes in concert with induction of systemic resistance in host plants.

## Materials and methods

### Genomic feature and prediction of genes involved in biocontrol of wheat blast by plant probiotic bacteria

Three plant probiotic bacterial strains, BTS-3, BTS-4 and BTLK6A were selected from screening 170 plant probiotic bacteria against a virulent strain of wheat blast fungus MoT previously isolated from various plants[2]. They were deposited in the freezer of the Department of Biotechnology of BSMRAU, Bangladesh before used in this experiment. The selected plant probiotics were isolated from the rice (BTS-3 and BTS-4) and wheat (BTLK6A) seeds[2]. The whole genomes sequences of three plant probiotic bacteria viz. BTS-3, BTS-4 and BTLK6A[3] were analysed to understand the underlying molecular mechanisms of wheat blast biocontrol by them. To reveal the genomic features, RAST annotation was performed with whole genome sequences of three plant probiotic strains. To find the number of subsystems in each strain, SEED viewer program was employed. For prediction of protein-coding genes, following RAST annotation, BLASTP (E-value <= $1e^{-113}$) search of annotated protein sequences with the sequences collected from UniProt knowledgebase was done to extract the similar protein sequence in three plant probiotic strains. Putative tRNAs were identified using ARAGORN (v 1.2.38) which included in parenthesis.

## Results and discussion

### General genomic features and taxonomy of the wheat blast biocontrol probiotic bacteria

The principal features of the BTLK6A, BTS-3 and BTS-4 genomes are summarized in Table 1 and Figure 1a-c. Taxonomic position of BTLK6A, BTS-3 and BTS-4 and were determined as *Bacillus amyloliquefaciens*, *B. subtilis,* and *B. amyloliquefaciens*, respectively[3].

Table 1. General features of the genomes of wheat blast biocontrol bacteria

| Genomic Features | BTS-3 | BTS-4 | BTLK6A |
|---|---|---|---|
| Closest species based on 16S rRNA gene sequence | *Bacillus subtilis* subsp. *subtilis* 168 | *Bacillus amyloliquefaciens* LL3 | *Bacillus amyloliquefaciens* LL3 |
| Genome size (bp) | 4,122,253 | 3,907,814 | 3,908,827 |
| G+C content (mol%) | 43.5 | 46.5 | 46.5 |
| N 50 (bp) | 1063829 | 2032688 | 1024542 |
| L 50 | 2 | 1 | 2 |
| Protein-coding sequences | 4272 | 3966 | 3968 |
| Percent of coding region | 88.0 | 89.4 | 89.3 |
| Average CDS size (bp) | 849 | 881 | 880 |
| Total number of RNAs | 113 | 98 | 98 |
| Number of Ribosomal RNAs | 17 | 14 | 17 |
| Number of tRNAs | 86 | 85 | 84 |
| Phage-associated genes | 13 | 13 | 57 |
| Number of Subsystems | 477 | 463 | 463 |

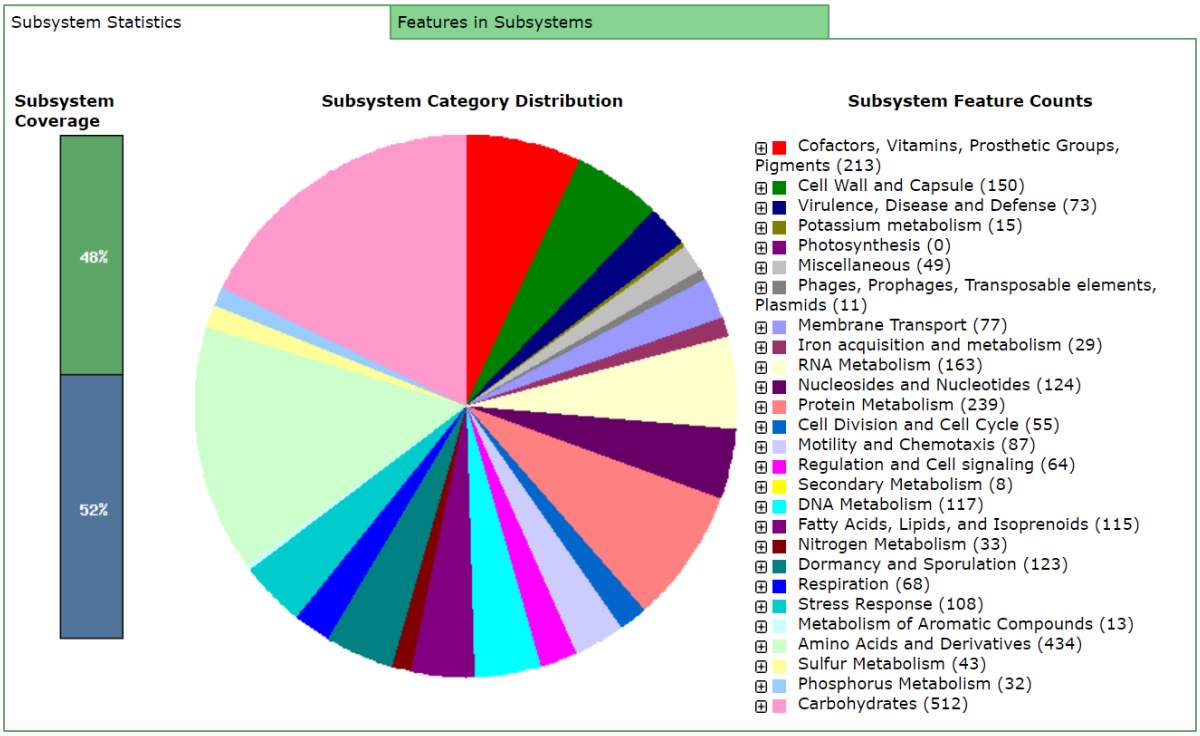


**Fig. 1a. *Bacillus subtilis* BTS-3 subsystem-features through SEED viewer**

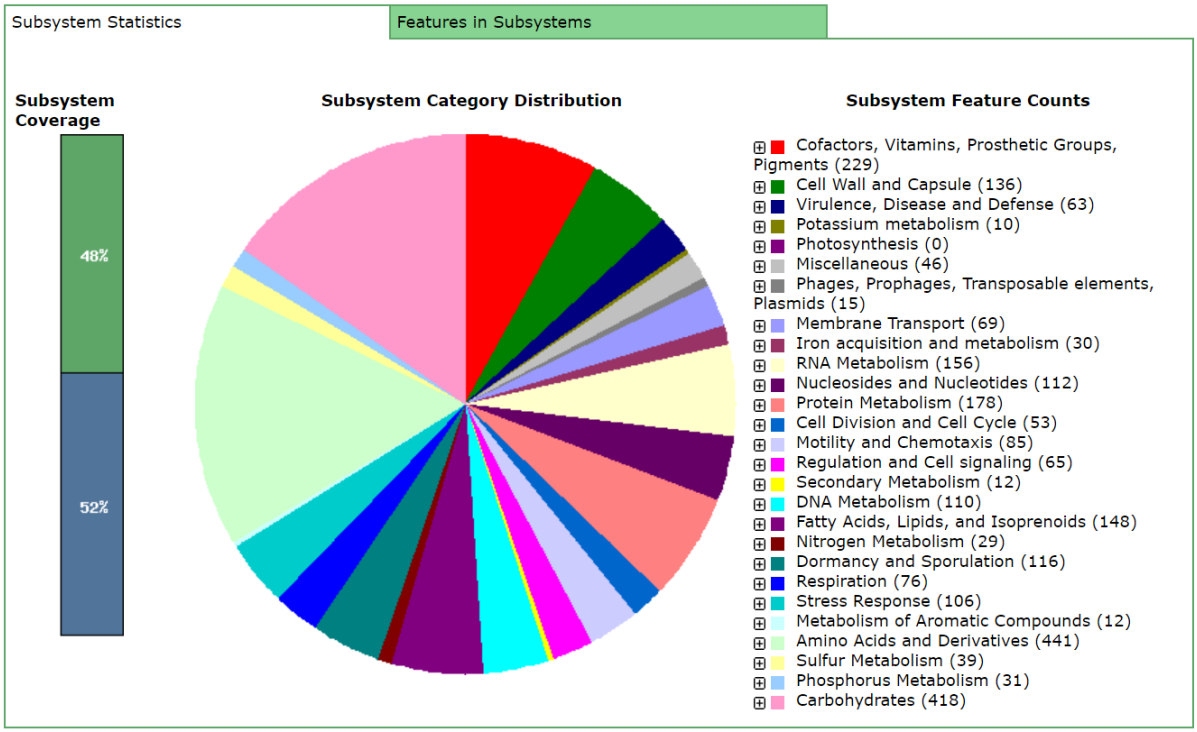


**Fig. 1b. *B. amyloliquefaciens* (BTS-4) subsystem-features through SEED viewer**

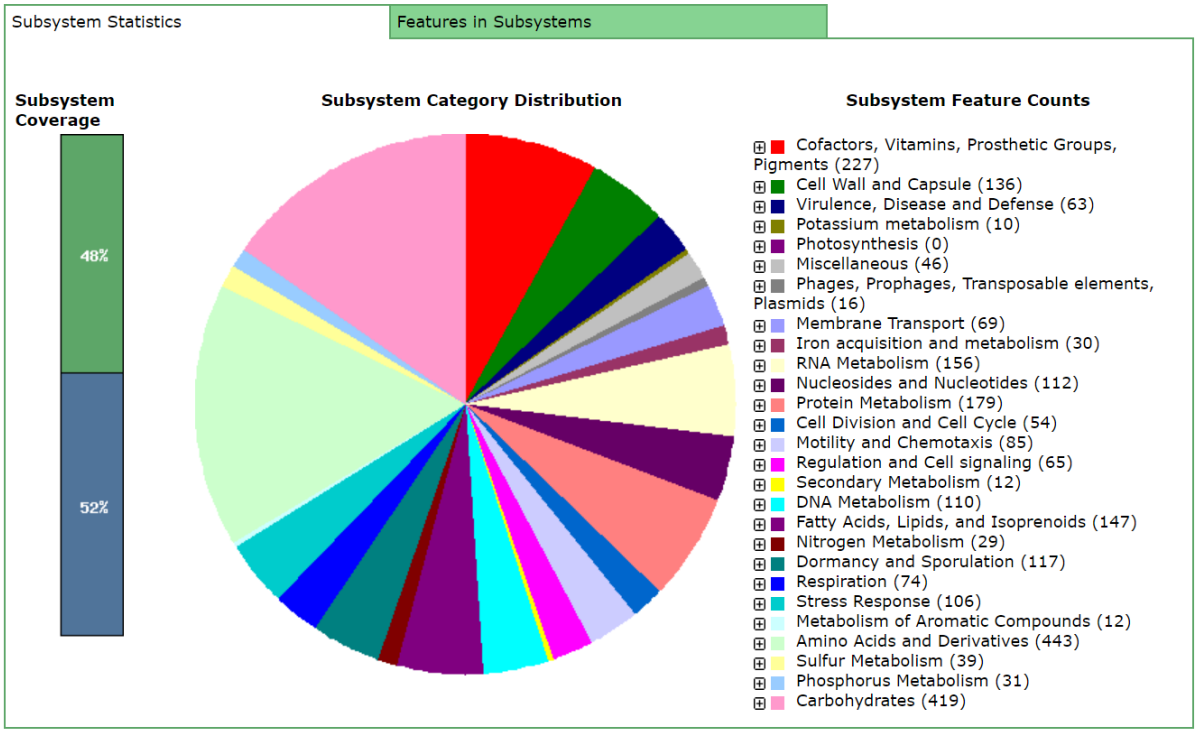


**Fig. 1c. *B. amyloliquefaciens* (BTLK6A): subsystem-features through SEED viewer**

### Gene clusters associated with biocontrol activities of the probiotic bacteria

*Bacillus* sp. possesses potential to synthesize bioactive secondary metabolites. The seven gene clusters that direct the synthesis of bioactive peptides and polyketides by both nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKS) (Table 2). Two (*mln* and *dfn*) are not found in *B. subtilis* (BTS-3) but rest five gene clusters (*bac, dhb, pps* and *npr*) are present in the genomes of all three *Bacillus* spp. (Table 2). The *bac* gene cluster is responsible for synthesis of bacillaene. *dbh* gene cluster is responsible for bacilibactin synthesis. In genome of BTS-4, there is a slight deviation in the homologue (*dhbABE*) compared to the genomes of other two bacteria (*dhbABCE* ). The presence of gene clusters in the genomes of three bacteria involved in the synthesis of diverse antimicrobial compounds suggest that antibiosis might be involved in biocontrol of wheat blast by these plant probiotics.

Table 2. Gene clusters involved in synthesis of secondary metabolites, antioxidant, cell wall degradation and volatile compound in plant probiotic bacteria

| **Compound** | **Enzyme** | **BTS-3** | BTS-4 | BTLK6A |
|---|---|---|---|---|
| **Gene clusters related to antibiotic production** | | | | |
| Bacillaene | PKS/NRPS | *baeBCDEGHIJLMN* | *baeBCDEGHIJLMN* | *baeBCDEGHIJLMN* |
| Macrolactin | PKS | Not present | *mlnABCDEFGHI* | *mlnABCDEFGHI* |

| | | | | |
|---|---|---|---|---|
| Difficidin | PKS | Not present | *dfnABCEFGHIJKLM* | *dfnABCEFGHIJKLM* |
| Bacilysin(siderophore) | NRPS | *bacABCDEFG* | *bacABCDEFG* | *bacABCDEFG* |
| Bacillibactin | NRPS | *dhbABCE* | *dhbABE* | *dhbABCE* |
| Plipastatin | | *ppsABCDE* | *ppsABCDE* | *ppsABCDE* |
| Putative peptide | NRPS | *nprE* | *nprE* | *nprE* |
| **Gene clusters related to antioxidant enzymes** | | | | |
| Superoxide dismutase | | *sodACF* | *sodACF* | *sodACF* |
| Glutathione peroxidase | | *bsaA* | *bsaA* | *bsaA* |
| Catalase | | *katAEX* | *katAEX* | *katAEX* |
| Thiol peroxidase | | *tpx* | *tpx* | *tpx* |
| **Gene cluster related to production of cell wall degradating enzymes** | | | | |
| Esterase | | *estAB* | *estAB* | *estAB* |
| Endoglucanase | | *eglS* | *eglS* | *eglS* |
| Beta-glucanase | | *bglS* | *bglS* | *bglS* |
| Pectatelyase | | *pelABC* | *pelAB* | *pelAB* |
| **Gene cluster related to volatile compound** | | | | |
| Acetoin | | *alsSD* | *alsSD* | *alsSD* |
| Hydrogen cyanide | | *Not found* | *hcnC* | *hcnC* |

According to genome analysis of three bacterial isolates, four gene clusters are involved in production of antioxidants. The *sod, bsa, kat* and *tpx* genes are responsible for production of superoxide dismutase, glutathione peroxidase, catalase and thiol peroxidase enzymes, respectively (Table 2). The *est, egl, bgl* and *pel* gene clusters in Bacilli are associated with enzymes involved in cell wall degradation. All four cell wall degradating enzymes were found in three biocontrol bacterial isolates. In *B. subtilis* (BTS-3), the pectatelyase enzyme homologue (*pelABC*) was slightly different from other two *B. amyloliquefaciens* (BTS-4 and BTLK6A) bacterial isolates homologue (*pelAB*) (Table 2). Acetoin (*Als*) gene cluster is available in all 3 bacterial genomes but genes responsible for production of hydrogen cyanide (*hcn*) only present in *B. amyloliquefaciens* but not in *B. subtilis* (Table 2).

Another interesting finding of our study is that genomes of all three wheat blast biocontrol bacteria possessed genes (acetolactate decarboxylase, *alsSD*) encoding biosynthesis of acetoin (3-hydroxy-2-butanone). Several lines of evidence suggest that acetoin is a powerful elicitor to trigger induced systemic resistance in plants[4,5]. Ruy et al. demonstrated that volatile acetoin produced by *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a significantly reduce infection of *Arabidopsis* seedlings by bacterial pathogen *Erwinia carotovora* subsp. *carotovora* compared with seedlings not exposed to bacterial volatiles before pathogen inoculation[6]. Using

transgenic and mutant lines of *Arabidopsis*, they demonstrated that the signalling pathway activated by volatile acetoin from GB03 is dependent on ethylene, albeit independent of the salicylic acid or jasmonic acid signalling pathways. The presence of gene clusters for biosynthesis of acetoin in all of our *Bacilli* suggesting that induced systemic resistance in wheat plants may also play a role in concert with antibiosis for biocontrol of wheat blast by these plant probiotic bacteria. Chowdhury et al. also showed that acetoin produced by PGPR is effective in biocontrol of plant pathogens and its expression in situ occurs during root colonization[7].

## Conclusion

Genomic analysis of the biocontrol Bacilli revealed that they possess genes responsible for biosynthesis of diverse antibiotics and enzymes with cell wall lytic functions. All three wheat blast biocontrol bacteria possessed genes (acetolactate decarboxylase, *alsSD*) encoding biosynthesis of acetoin (3-hydroxy-2-butanone) which is known to induce systemic resistance (ISR) in host plant. Our results suggest that probiotic bacterial strains, BTS-3, BTS-4 and BTLK6A protect wheat seedlings from blast fungus directly by the production and secretion of inhibitory secondary metabolites and lytic enzymes to the pathogen and indirectly through induction of ISR in wheat plants. Further experiment (such as mutants etc.) is needed to confirm specific role(s) of the predicted genes on biocontrol of wheat blast by the respective plant probiotic bacteria.

## Funding Source

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