**Supplementary material**

Type of the Paper (Article)

Lipopeptides Produced by Bacillus spp.: Detection of Genes and Meta-analysis of Their Activity Against Phytopathogenic Fungi

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**Table S2.** Oligonucleotides used for detection of lipopeptide biosynthesis genes.[1]

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Lipopeptides** | **gene** | **First** | **Sequence (5’—3’)** | **Tm (°C)** | **Size (pb)** |
| Iturin A | *ituA* | ITUD1F  ITUD1R | GATGCGATCTCCTTGGATGT  ATCGTCATGTGCTGCTTGAG | 60 | 647 |
| Fengicine | *fenD* | FENDIF  FEND1R | TTTGGCAGCAGGAGAAGTTT  GCTGTCCGTTCTGCTTTTTC | 62 | 964 |
| Surfactin | *srfA* | SUR3F  SUR3F | ACAGTATGGAGGCATGGTC  TTCCGCCACTTTTTCAGTTT | 57 | 441 |

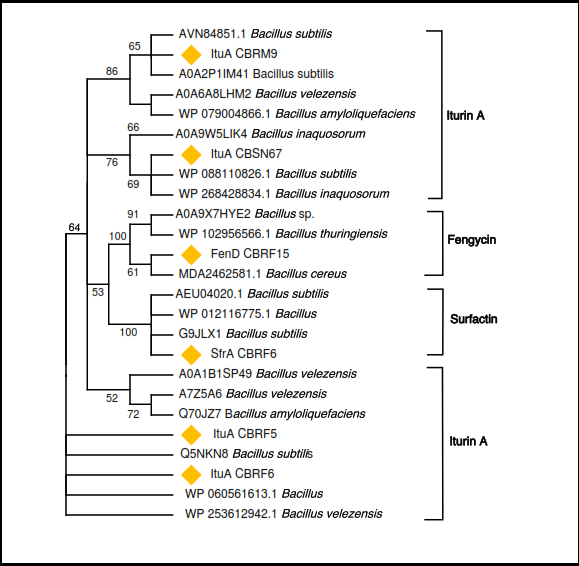
Tm: Melting Temperature; pb: Base pairs.

**Table S1.** General summary of data sources for Meta-analysis.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species** | **Lipopeptide** | **Variable** | **Dose** | **Reference** |
| *F. graminearum* | Iturin | ICM (%) | 5, 10, 25, 50 μg/mL | [2] |
| *B. maydis* | Iturin | Severity | 50, 100, 300,500 μg/mL | [3] |
| *F. moniliforme* | Surfactin | ICM (%) | 6.25, 12.5, 25, 50, 100, 200 μg/mL | [4] |
| *R. stolonifer* | Fengicine | ICM (%) | 25, 50, 100, 200, 400, 600 μg/mL | [5] |
| *F. oxysporum* | Fengicine | ICM (%) | 12.5, 25, 50 μg/mL | [6] |
| *C. gloeosporioides* | Iturin | Severity | 10.50μg/mL | [7] |
| *C. gloeosporioides* | Surfactin | Severity | 10.50μg/mL | [7] |
| *Z. tritici* | Surfactin | Severity | 100 μg/mL | [8] |
| *Z. tritici* | Fengicine | Severity | 100 μg/mL | [8] |
| *A. flavus* | Surfactin | ICM (%) | 20, 40, 80 μg/mL | [9] |
| *A. alternata* | Iturin | ICM (%) | 60 μg/mL | [10] |
| *B. cinerea* | Iturin | ICM (%) | 60 μg/mL | [10] |
| *C. gloeosporioides* | Iturin | ICM (%) | 60 μg/mL | [10] |
| *F. oxysporum* | Iturin | ICM (%) | 60 μg/mL | [10] |
| *R. solani* | Iturin | ICM (%) | 60 μg/mL | [10] |
| *P. oryzae* | Iturin | Severity | 500 μg/mL | [11] |
| *P. oryzae* | Surfactin | Severity | 500 μg/mL | [11] |
| *S. oryzae* | Iturin | Severity | 500 μg/mL | [11] |
| *S. oryzae* | Surfactin | Severity | 500 μg/mL | [11] |
| *B. cinerea* | Iturin | Severity | 250, 500 μg/mL | [11] |
| *B. cinerea* | Surfactin | Severity | 250 μg/mL | [11] |
| *P. infestans* | Iturin | Severity | 500 μg/mL | [11] |
| *P. infestans* | Surfactin | Severity | 500 μg/mL | [11] |
| *P. trichina* | Iturin | Severity | 500 μg/mL | [11] |
| *C. gloeosporioides* | Iturin | Severity | 250, 500 μg/mL | [11] |
| *C. gloeosporioides* | Surfactin | Severity | 250, 500 μg/mL | [11] |

**Phylogenetic analysis**

The phylogenetic tree was constructed using the deduced amino acid sequence of iturin A, fengycin, and surfactin and their homologous protein sequences retrieved from the NBCI and UniProt database. The phylogenetic tree was constructed using the Maximum Likelihood method based on Jones-Taylor-Thornton (JTT) substitution using 1000 bootstrap through MEGA X software.[12,13] Sites with less than 95% coverage such as gaps, missing data, and ambiguous bases were partially eliminated. Amino acid sequences were aligned using MUSCLE with default MEGA X parameters. The substitution model was estimated using the best-fit substitution model (ML) function in the MEGA X software.



**Figure S1.** Phylogenetic tree of the deduced amino acid sequence of iturin A (ituA), surfactin (srfA), and fengycin (FenD) from Bacillus strains (CBRF5, CBRF6, CBRM9 CBRF15) and their homologous protein sequences. The accession number of the proteins used is indicated. Protein sequences were retrieved from the NCBI and UniProt database.

**References**

1. Ramarathnam, R.; Bo, S.; Chen, Y.; Fernando, W.G.D.; Xuewen, G.; De Kievit, T. Molecular and Biochemical Detection of Fengycin- and Bacillomycin D-Producing Bacillus Spp., Antagonistic to Fungal Pathogens of Canola and Wheat. *Can. J. Microbiol.* **2007**, *53*, 901–911, doi:10.1139/W07-049/ASSET/IMAGES/LARGE/W07-049F4.JPEG.

2. Gong, A.D.; Li, H.P.; Yuan, Q.S.; Song, X.S.; Yao, W.; He, W.J.; Zhang, J.B.; Liao, Y.C. Antagonistic Mechanism of Iturin A and Plipastatin A from Bacillus Amyloliquefaciens S76-3 from Wheat Spikes against Fusarium Graminearum. *PLoS One* **2015**, *10*, e0116871, doi:10.1371/JOURNAL.PONE.0116871.

3. Ye, Y. feng; Li, Q. qin; Fu, G.; Yuan, G. qing; Miao, J. hua; Lin, W. Identification of Antifungal Substance (Iturin A2) Produced by Bacillus Subtilis B47 and Its Effect on Southern Corn Leaf Blight. *J. Integr. Agric.* **2012**, *11*, 90–99, doi:10.1016/S1671-2927(12)60786-X.

4. Jiang, J.; Gao, L.; Bie, X.; Lu, Z.; Liu, H.; Zhang, C.; Lu, F.; Zhao, H. Identification of Novel Surfactin Derivatives from NRPS Modification of Bacillus Subtilis and Its Antifungal Activity against Fusarium Moniliforme. *BMC Microbiol.* **2016**, *16*, 1–14, doi:10.1186/S12866-016-0645-3/FIGURES/9.

5. Tao, Y.; Bie, X. mei; Lv, F. xia; Zhao, H. zhen; Lu, Z. xin Antifungal Activity and Mechanism of Fengycin in the Presence and Absence of Commercial Surfactin Against Rhizopus Stolonifer. *J. Microbiol.* **2011**, *49*, 146–150, doi:10.1007/S12275-011-0171-9/METRICS.

6. Zhao, P.; Quan, C.; Wang, Y.; Wang, J.; Fan, S. Bacillus Amyloliquefaciens Q-426 as a Potential Biocontrol Agent against Fusarium Oxysporum f. Sp. Spinaciae. *J. Basic Microbiol.* **2014**, *54*, 448–456, doi:10.1002/JOBM.201200414.

7. Yamamoto, S.; Shiraishi, S.; Suzuki, S. Are Cyclic Lipopeptides Produced by Bacillus Amyloliquefaciens S13‐3 Responsible for the Plant Defence Response in Strawberry against Colletotrichum Gloeosporioides? *Lett. Appl. Microbiol.* **2015**, *60*, 379–386, doi:10.1111/LAM.12382.

8. Mejri, S.; Siah, A.; Coutte, F.; Magnin-Robert, M.; Randoux, B.; Tisserant, B.; Krier, F.; Jacques, P.; Reignault, P.; Halama, P. Biocontrol of the Wheat Pathogen Zymoseptoria Tritici Using Cyclic Lipopeptides from Bacillus Subtilis. *Environ. Sci. Pollut. Res.* **2018**, *25*, 29822–29833, doi:10.1007/S11356-017-9241-9/FIGURES/6.

9. Mohammadipour, M.; Mousivand, M.; Jouzani, G.S.; Abbasalizadeh, S. Molecular and Biochemical Characterization of Iranian Surfactin-Producing Bacillus Subtilis Isolates and Evaluation of Their Biocontrol Potential against Aspergillus Flavus and Colletotrichum Gloeosporioides. *Can. J. Microbiol.* **2009**, *55*, 395–404, doi:10.1139/W08-141/ASSET/IMAGES/W08-141T3H.GIF.

10. Dang, Y.; Zhao, F.; Liu, X.; Fan, X.; Huang, R.; Gao, W.; Wang, S.; Yang, C. Enhanced Production of Antifungal Lipopeptide Iturin A by Bacillus Amyloliquefaciens LL3 through Metabolic Engineering and Culture Conditions Optimization. *Microb. Cell Fact.* **2019**, *18*, 1–14, doi:10.1186/S12934-019-1121-1/TABLES/3.

11. Kong, H.G.; Lee, H.J.; Bae, J.Y.; Kim, N.H.; Moon, B.J.; Lee, S.W. Spatial and Temporal Distribution of a Biocontrol Bacterium Bacillus Licheniformis N1 on the Strawberry Plants. *Plant Pathol. J.* **2010**, *26*, 238–244, doi:10.5423/PPJ.2010.26.3.238.

12. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549, doi:10.1093/MOLBEV/MSY096.

13. Stecher, G.; Tamura, K.; Kumar, S. Molecular Evolutionary Genetics Analysis (MEGA) for MacOS. *Mol. Biol. Evol.* **2020**, *37*, 1237–1239, doi:10.1093/MOLBEV/MSZ312.