

Seed-Associated Bacterial Endophytes Enhance Barley Growth and Mitigate *Fusarium graminearum* Infection

Aisha Parween, Vidyanath Jha

Abstract: This study explores the potential of bacterial seed endophytes in promoting barley growth and suppressing *Fusarium graminearum*, the causative agent of Fusarium head blight (FHB). Culture-dependent and molecular approaches identified *Bacillus* species as predominant seed inhabitants. Selected isolates demonstrated strong growth-promoting effects on root and shoot elongation, while certain strains also inhibited fungal growth in vitro. These findings highlight the dual role of barley seed endophytes as both plant growth promoters and biocontrol agents, offering sustainable alternatives to chemical inputs.

Keywords: Barley, Endophytes, Bacillus, Bio-priming, Fusarium head blight, Biocontrol

1.Introduction

Barley is a vital cereal crop often threatened by Fusarium head blight (FHB), a destructive disease that can lead to severe yield losses and contamination with harmful mycotoxins. Conventional reliance on chemical fertilizers and fungicides raises environmental concerns, thereby necessitating eco-friendly approaches. Seeds harbor diverse microbial communities, including endophytic bacteria, that play key roles in nutrient mobilization, stress tolerance, and pathogen defense. Harnessing such microbes through bio-priming offers promising strategies to enhance crop resilience and productivity.

Earlier works reported that some beneficial microbes demonstrated both antifungal and plant growth promoting properties, and are attractive candidates for improving plant growth and boosting plant resistance to pathogen attack. Taking into consideration the above-mentioned benefits of bacterial seed endophytes, our goal was to evaluate malting barley seeds grown in different environments and identify bacterial endophytes with

Dual function roles in promoting seedling establishment and suppressing FHB disease.

2.Materials and Methods

Barley seeds from multiple locations were surface-sterilized and analyzed for culturable bacterial endophytes. Isolates were identified based on morphological and molecular characterization using 16S rRNA sequencing. Seed bio-priming assays were conducted by soaking seeds in bacterial suspensions, followed by growth chamber experiments to evaluate root and shoot elongation. Antagonistic assays were performed in vitro against *Fusarium graminearum* using dual culture techniques to assess inhibition zones and growth suppression.

Molecular characterization of bacterial isolates

Genomic DNA was extracted from bacterial cells pelletized from liquid cultures using Zymo Research Quick DNA fungal/Bacterial Miniprep Kit (Irvine, California, USA), following the manufacturers protocol. The 16 S rRNA genes

of the bacterial strains were amplified by PCR using the universal primer pair 27 F (5' - A G A G T T T G A T C- MTGGCTCAG- 3') and 1492R (5' - G G T T A C C T T G T T A C-GACTT- 3'). The amplified PCR products were purified from the bands (approx;1500 bp) using Gel and PCR Clean-Up System (Promega, USA), and sequenced using long read amplicon sequencing. Identical bacterial sequences were retrieved using BLAST.

3.Results

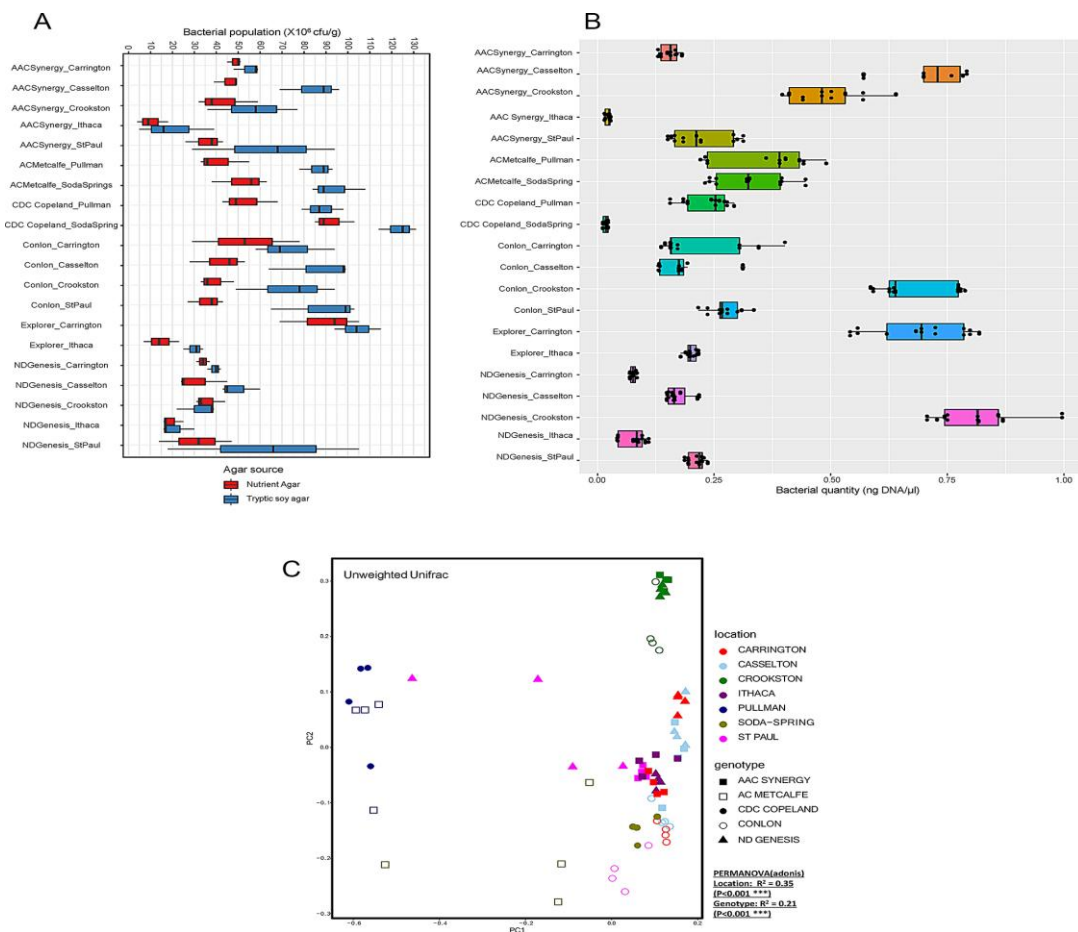
Analysis revealed that *Bacillus subtilis*, *B. licheniformis*, and *B. pumilus* were dominant endophytes in barley seeds. Geographic location influenced microbial diversity, with significant differences in bacterial abundance across sites. Seed priming experiments showed that a subset of bacterial isolates enhanced both root and shoot growth by more than 10%. Notably, five isolates exhibited strong antifungal activity against *F. graminearum*, reducing fungal growth significantly in vitro.

4.Discussion

The results demonstrate the dual functionality of barley seed endophytes as plant growth promoters and biocontrol agents. Location-specific variation in microbial communities suggests that environmental conditions play a crucial role in shaping seed microbiota. The predominance of *Bacillus* species is consistent with their known ability to produce phytohormones, antimicrobial compounds, and enhance plant systemic resistance. These findings support the potential application of seed-associated endophytes in sustainable agriculture, reducing dependence on synthetic chemicals.

5.Conclusion

Seed-associated bacterial endophytes represent promising candidates for eco-friendly agricultural practices. Their ability to enhance barley seedling growth while simultaneously suppressing *Fusarium* infections positions them as valuable bioinoculants. Future field-based evaluations are essential to validate their effectiveness under natural growth conditions.



References

- [1] Alisaac E, Mahlein AK. Fusarium Head Blight on Wheat: Biology and Integrated Management. Toxins, 2023.
- [2] Ashwini K, Suman A, Sharma P, Singh PK, Gond S, Pathak D. Seed endophytic Bacterial profiling from wheat varieties of contrasting heat sensitivity. Front Plant Sci. 2023; 14:1101818.
- [3] Gowtham H, Murali M, Singh SB, Lakshmeesha T, Murthy KN, Amruthesh K, et al. Plant growth promoting rhizobacteria-Bacillus amyloliquefaciens improves plant growth and induces resistance in Chilli against anthracnose disease. Biol Control. 2018; 126:209–17.
- [4] Herrera SD, Grossi C, Zawoznik M, Groppa MD. Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of Fusarium Graminearum. Microbiol Res. 2016; 186:37–43.
- [5] Naziya B, Murali M, Amruthesh KN. Plant growth-promoting fungi (PGPF) instigate plant growth and induce disease resistance in Capsicum annuum L. upon infection with Colletotrichum capsici (Syd.) Butler & Bisby. Biomolecules. 2019;10(1):41.
- [6] Oerke EC, Dehne HW. Crop losses and control. Elsevier, 2012
- [7] Rai PK et al. Role of plant growth-promoting rhizobacteria in sustainable agriculture. Elsevier, 2020
- [8] Sun, L., Wang, X., Li, Y., 2016. Increased plant growth and copper uptake of host and nonhost plants by metal-resistant and plant growth-promoting endophytic bacteria. Int. J. Phytoremed. 18, 494–501.
- [9] Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N., Barac, T., Vangronsveld, J., van der Lelie, D., 2009. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl. Environ. Microbiol. 75, 748–757.
- [10] Terakado-Tonooka, J., Ohwaki, Y., Yamakawa, H., Tanaka, F., Yoneyama, T., Fujihara, S., 2008. Expressed nifH genes of endophytic bacteria detected in field-grown sweet potatoes (*Ipomoea batatas* L.). Microbes Environ. 23 (1), 89–93.
- [11] Thomas, P., Upreti, R., 2014. Testing of bacterial endophytes from non-host sources as potential antagonistic agents against tomato wilt pathogen Ralstonia solanacearum. Adv. Microbiol. 4, 656.
- [12] Van Der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11, 296–310.
- [13] Visioli, G., D'Egidio, S., Vamerali, T., Mattarozzi, M., Sanangelantoni, A.M., 2014. Culturable endophytic bacteria enhance Ni translocation in the hyperaccumulator Nocca caerulescens. Chemosphere 117, 538–544.
- [14] Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. 52 (suppl 1), 487–511