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Whole Genome Analysis of Biocontrol Agents Bacillus halotolerans KFD Uncovers Its Molecular Arsenal Against the Bayoud Pathogen Fusarium oxysporum f.sp albedinis and Its Potential to Enhance Plant Growth

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Abstract : The bayoud disease stands as a serious threat to date palm cultivation and production in North Africa, particularly in Morocco. Different attempts to manage the disease using breeding techniques or chemical pesticides did not yield promising results, leading farmers to burn infected trees to limit the pathogen propagation. As an alternative, biocontrol agents constitute an eco-friendly solution and a promising approach to this problem. In this frame, we isolated different bacteria from date palm roots that were screened for their potential to inhibit the pathogen Fusarium oxysporum f.sp. albedinis (Foa). Out of forty tested isolates, one isolate showed particularly promising results against Foa and exhibited as well broad-spectrum antifungal properties against other phytopathogenic fungi namely Fusarium solani, F. oxysporym f.sp. lycopersici, F. proliferatum, Phytophtora infestans, and Botrytis cinerea. Moreover, this isolate was tested for plant growth-promoting (PGP) traits and the result showed that it could solubilize inorganic phosphate, produce siderophores, IAA, ACC deaminase and fix nitrogen. We also conducted greenhouse assay to evaluate the protective effect of our isolate. The result showed that our isolate effectively protected date palm seedlings against Foa. To extend our understanding of the molecular mechanisms underlying the antifungal and PGP features, we sequenced and characterized the genome. The genomic characterization showed that our isolate has a genome size of approximately 3.9 MB and belongs to Bacillus halotolerans. The Average nucleotide identity showed that our isolate was a new strain which we named B. halotolerans KFD. We annotated 11 secondary metabolites gene clusters encompassing six known antifungal clusters namely bacillibactin, bacilysin, bacillaene, fengycin, surfactin, and plipastatin. Moreover, we identified genes that encode carbohydrate-active enzymes among which GH18 and GH23 directly involved in chitinase activities and the GH30, GH5 and GH26 subfamilies implicated in the degradation of glucan in fungal cell walls. The prediction of secreted proteins reported the secretion of antifungal products such as plipastatine and bacillolysin, and enzyme implicated in fungal cell wall degradation such as N-Acetylglucosaminidase and β-glucanase. The comparative genomic analysis showed that B. halotolerans KFD contained 146 unique genes, among which was a gene encoding plipastatin, a cyclic lipopeptide known to have antifungal properties against plant pathogens. The screening of genes linked to plant growth promotion identified genes involved in phosphate metabolism (pstA, pstB, pstC, pstS, ykaB, ykaA, cysP, yjkB, yqgI, yqgH, phoP, and phoR), IAA production (trpA, trpB, trpC, trpD, trpE, trpF, and trpP), and in nitrogen fixation (yutI). Moreover, we predicted 12 genes implicated in siderophore production and iron transport ybdZ, dhbE, yvrB, fhuG, fhuB, yfmE, yfmD, yfiZ, yfhA, yclN, yclO, and feuC. Our results show that Bacillus halotolerans KFD represents a potential biocontrol agent that could be used to manage bayoud disease and promote the growth of date palm. To the best of our knowledge, our study is the first to isolate and describe the genomic features of a B. halotolerans strain from the rhizosphere of date palm.

Keywords : biocontrol agents, date palm, Fusarium oxysporum f.sp. albedinis, Bayoud disease, Bacillus halotolerans, Antifungal, Genome analysis, PGPR
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