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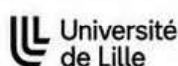
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## Phenotypic and Genotypic Characterization, Synbiotic Formulation, and Agronomic Potential of Multifunctional Probiotic *Bacillus subtilis* B3VCR2

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### Abstract

*Bacillus subtilis* B3VCR2 was isolated from soumbala (fermented *Parkia biglobosa* condiment, Burkina Faso) and formally identified by 16S rRNA genome sequencing, MALDI-TOF MS analysis, and thermophysical fingerprinted. It was then characterised as a multifunctional plant growth-promoting bacterium with probiotic potential via polyphasic characterisation combining phenotypic assays and whole-genome sequencing. Species identity was confirmed (ANI >98%; dDDH >89%) and MLST analysis revealed a novel allele at the *purH* locus. Phenotypically, *B. subtilis* B3VCR2 was non-haemolytic, susceptible to six of seven antibiotics tested with intrinsic streptomycin resistance only, exhibited antioxidant activity, enzymatic activities (amylase, cellulase, protease), emulsifying activities, nitrogen fixation capacity, phosphorus solubility activity, and antifungal activity against *Fusarium oxysporum*. By analysing cell-free supernatants, antimicrobial lipopeptides (fengycin & surfactin), organic acids, indole-3 acetic acid, volatile compounds such as  $\beta$ -Carboline 2-methyl-, 1H-pyrido [2,3-*b*]indole, and n-hexadecanoic acid, and exopolysaccharides were detected. The draft genome (43.72% G+C; 99.59% completeness) harboured 14 biosynthetic gene clusters (BGCs), including operons for surfactin, fengycin, bacilysin, bacillaene, subtilosin A, subtilomycin, and bacillibactin. Plant growth-promoting genes encoding auxin biosynthesis (indole-3-pyruvate pathway), phosphate solubilisation (*phoA*, *gdh*), siderophore production (*dhb* operon), and nitrogen assimilation (GS-GOGAT pathway) were identified alongside stress-tolerance gene sets. Field trials in Burkina Faso evaluated *B. subtilis* B3VCR2 combined with fructooligosaccharides (FOS) as a synbiotic soil amendment for tomato cultivation across three intercropping systems. One-way ANOVA revealed highly significant treatment effects on available phosphorus ( $p < 0.001$ ), C/N ratio ( $p < 0.001$ ), and organic carbon ( $p = 0.002$ ). The *B. subtilis* B3VCR2 treatment increased available phosphorus and delivered tomato greater yields (18,6 kg/ha) over the unamended control (2,9 kg/ha) and provided comparable results to chemical biopesticide Fertineem treatment (21,0 kg/ha). These findings establish *B. subtilis* B3VCR2 as a safe, genomically novel, and agronomically effective bio-inoculant for sustainable peri-urban agriculture, validating traditional fermented foods as reservoirs of agronomically useful *Bacillus* strains.

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