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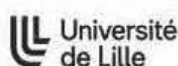
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African Food and non-Food products as Sources of Functional *Bacillus* Probiotics for Sustainable Animal Feeding.

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Abstract

The progressive restriction of antibiotics in livestock production has intensified the search for sustainable probiotic alternatives compatible with the One Health framework. In this study, food and non-food products from Morocco and Burkina-Faso were investigated as sources of functional *Bacillus* probiotics for animal nutrition. A total of 42 bacterial morphotypes were isolated from soils associated with orange trees, silage, and soumbala (West-Africa fermented food). Taxonomic identification based on 16S rRNA gene sequencing and MALDI-TOF Mass Spectrometry analysis revealed 14 isolates (E6, E7, E8, E9, E10, E25, E26, B2VCR1, B2VCR2, B2VCR3, B3VCR2, B10VCR1, SO11, and BGma), mainly affiliated with the *Bacillus subtilis* complex, including *Bacillus subtilis*, *Bacillus mojavensis*, *Bacillus halotolerans*, and *Bacillus velezensis*. These isolates were then subjected to an in vitro screening strategy designed to select strains combining multiple probiotic mechanisms relevant to commercial application. The screening assessed six functional traits: acid tolerance, bile salt resistance, auto-aggregation, cell-surface hydrophobicity, antimicrobial activity, and sporulation capacity. Marked inter-strain variability was observed. Soil-derived isolates E8 and E9, silage-derived isolate E26, and soumbala-derived isolates B2VCR2, B2VCR1, and B2VCR3 showed strong resistance to simulated gastrointestinal stress, including growth in 0.3% (w/v) bile salts. Silage-derived isolates E25 and E26 also displayed excellent survival at pH 2.0. High auto-aggregation levels, exceeding 70% for most strains, indicated promising adhesion potential. Cell-free supernatants from E25, E26, E7, and E9 produced large inhibition zones against *Salmonella enterica* and *Aeromonas hydrophilia* suggesting the secretion of bioactive antimicrobial metabolites. Sporulation assays confirmed the ability of E9, E25, B2VCR1, and B3VCR2 to form heat-resistant spores efficiently. Multivariate analyses identified E25, E26, E6, and E7 as the most promising candidates. These strains combine stress tolerance, antimicrobial activity, adhesion potential, and sporulation efficiency, making them strong prospects for next-generation animal probiotics. Overall, African agroecosystems emerge as valuable reservoirs for developing sustainable *Bacillus*-based solutions for livestock production.

Keywords: *Bacillus*; Probiotic; Antibacterial activity; Animal Feed.