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MOLECULAR IDENTIFICATION AS A CRITICAL STEP IN SCREENING SOIL BACTERIA FOR PGPR USE

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ABSTRACT

In recent years, increasing attention has been directed toward the isolation and characterization of beneficial soil bacteria with plant growth-promoting (PGPR) properties and/or biocontrol potential. These microorganisms play a crucial role in sustainable agriculture by enhancing nutrient availability, stimulating plant development, and suppressing soil-borne pathogens through various direct and indirect mechanisms. Research efforts in this area typically begin with the screening of bacterial isolates for specific functional traits, including siderophore production (which enables iron acquisition), synthesis of phytohormones such as indole-3-acetic acid (IAA), phosphate solubilization (improving phosphorus uptake), and ACC deaminase activity (which mitigates plant stress caused by ethylene accumulation). In addition, many studies assess the antagonistic potential of isolates against phytopathogenic fungi, as this is a key indicator of biocontrol capability. Collectively, these functional attributes form the foundation for selecting promising candidates for bioinoculant development and further field application. However, initial screening is often conducted prior to taxonomic identification, which poses a potential biosafety issue if isolates belong to species that may be pathogenic to humans, plants, or other organisms. The aim of this study was to investigate the presence of potentially pathogenic bacteria in soil samples used for the isolation of PGPR candidates. Thirteen soil samples were collected from different locations across Serbia. Bacteria were isolated using the standard serial dilution plating method on nutrient agar, without pre-selection, in order to capture the full diversity of cultivable strains. All isolates were subjected to 16S rDNA sequencing for taxonomic identification. Identification of isolates was performed based on the 16S RNA gene sequences by using P0 (5'-GAGAGTTTGATCCTGGCTCAG-3') and P6 (5'-CTACGGCTACCTTGTACGA-3') primers, while the sequencing was done by using commercial service (Macrogen Europe). Based on the obtained sequences, the Neighbour-Joining (NJ) phylogenetic tree was constructed in Mega V.11 program. Molecular analyses revealed the presence of *Lysinibacillus fusiformis*—a species with literature-documented opportunistic pathogenic potential—in two out of thirteen samples. According to published studies, strains of *L. fusiformis* have been associated with human diseases such as tropical ulcers and dermal or respiratory infections. No potentially pathogenic species were detected in the remaining eleven samples. These findings underscore the importance of molecular identification of soil isolates intended for agricultural application, especially in the context of bioinoculant development. Genetic characterization at an early stage of screening can serve as an essential tool for ensuring the biosafety and regulatory compliance of microbial-based products. In conclusion, when identifying such species additional analyses are required in order to evaluate the safety of its further use.

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Keywords: PGPR, soil bacteria, 16S rDNA sequencing, *Lysinibacillus fusiformis*, biosafety, microbial inoculants, opportunistic pathogens, sustainable agriculture