HRMS ANALYSIS OF SURFACTIN-TYPE COMPONENTS PRODUCED BY *BACILLUS* STRAINS ISOLATED FROM ENVIROMENTAL SAMPLES

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Abstract
Surfactin-type components produced by *Bacillus* species are cyclic lipopeptides with broad potential across environmental sustainability, agriculture, and environmental protection. In addition to their ecological relevance, these compounds also exhibit practical bioactivities such as antibacterial, antifungal, antiviral, and antitumor properties. In this study, we successfully performed the taxonomic identification of 40 *Bacillus* strains isolated from fungal compost. The surfactin production by each strain, as well as the individual surfactin variants, was examined by High-Resolution Mass Spectrometry (HRMS). Of the 40 *Bacillus* strains, 18 were found to produce surfactins, representing members of the species *B. subtilis*, *B. halotolerans*, *B. licheniformis*, and *B. velezensis*. A total of 109 distinct surfactin variants were identified among the isolates. Following the spectrometric analysis, biological assessments were performed to determine the antimicrobial activity of the surfactin producing strains against phitopathogenic bacteria and fungi. *B. subtilis* strains were generally effective against phytopathogenic bacteria. For phytopathogenic fungi, *B. halotolerans* strains demonstrated the strongest activity. In general, our findings on the production of surfactin by *Bacillus* isolates provide a solid foundation for the identification of promising candidates for future biocontrol applications.

Introduction
The importance of studying surfactin production by *Bacillus* species is due to their role in environmental sustainability [1], health and medical uses [2], agriculture and environmental protection [3]. The use of biosurfactants at low concentrations exceeds the effectiveness of synthetic surfactants, they are more easily degraded and more stable under extreme conditions [4]. These properties make them suitable for industrial use. The study of their structure may contribute to the development of synthetic alternative surfactants with less environmental impact due to their easier degradation. In medicine, the antimicrobial effects of surfactins are exploited. The use of surfactins as antibiotics may offer an alternative to increasing antibiotic resistance [5]. Surfactins are also used in cancer research to efficiently deliver drugs to cancer cells [6]. Their use as biopesticides in agriculture can reduce the use of chemical pesticides, and their ability to solubilise and mobilise contaminants in soil and water can be used in bioremediation projects [7]. In the food industry, surfactins can act as natural emulsifiers in foods, improving texture, stability and shelf-life [8]. Research in this area focusses on the development of natural, safe food additives that can replace synthetic emulsifiers, often derived from chemicals or genetically modified organisms.

Experimental

For the strains taxonomic determinations, the *rpoB* gene sequences were amplified by PCR from purified template DNA of the bacteria. The surfactin production of each strain was examined by mass spectrometry. The individual surfactin variants were detected in each *Bacillus* strain by HRMS. The analysis of the surfactin variants was based on the characteristic fragmentation patterns of surfactin molecules. In the HRMS method surfactin molecules were analysed according to the mass of the sodium adduct from of the molecular ion (*m/z* 1016, *m/z* 1030, *m/z* 1044, *m/z* 1058, *m/z* 1072, *m/z* 1086, *m/z* 1100, *m/z* 1114).
Biological assays were carried out to determine the antimicrobial activity of surfactin producing strains. The inhibition zones were measured against phytopathogenic fungi and bacteria.

Results and discussion

We successfully performed the taxonomic identifications of 40 *Bacillus* strains form environmental samples. Following the taxonomic characterization, we carried out a detailed investigation of surfactin production by these strains. Of the 40 *Bacillus* strains, 18 were found to produce surfactins, representing members of the species *B. subtilis, B. halotolerans, B. licheniformis* and *B. velezensis*. A total of 109 distinct surfactin variants were identified among the isolates. The strains those produced the highest amount of surfactin were members of the *B. halotolerans* and *B. licheniformis* species.
Against the phytopathogenic bacteria, the *B. subtilis* strains were generally effective, although minor inhibition zones were observed. For the phytopathogenic fungi, most of the strains exhibited inhibition zones, with the largest inhibition were produced by members of the *Bacillus halotolerans* species.

Conclusion

The taxonomic identification of 40 *Bacillus* strains was performed using *rpoB* gene sequences. Through HRMS analysis, 18 surfactin producing strains were identified. A total of 109 distinct surfactin variants were identified among the isolates. Against phytopathogenic bacteria, *B. subtilis* strains were generally effective; for phytopathogenic fungi, *B. halotolerans* strains were the most effective.

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