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BIOSURFACTANT PROSPECTION IN MARINE Bacillus sp. STRAINS USING A COMPUTATIONALLY ASSISTED REVERSED DROP-COLLAPSE ASSAY

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Surfactants are compounds that reduce surface tension between two different phases. Also known as tensoactives, the increasing demand such substances is driven by their broad applications across chemical, pharmaceutical, food, and environmental industries. Among these compounds, microbial biosurfactants stand out due to key advantages such as high biodegradability, low toxicity, biocompatibility, and stability under varying pH, temperature, and salinity. The *Bacillus* genus is widely recognized as a biosurfactant producer; however, its marine species remain underexplored despite their significant biotechnological potential. The drop-collapse assay amply applied in screening protocols for the presence of surfactants in liquid samples; however, data analysis can be challenging due to the lack of methodization. Herein, this study evaluated six marine *Bacillus* strains from the MicroMarin culture collection — a repository of bacteria isolated from sediments and marine invertebrates collected along the Brazilian coast and islands — along with a reference strain (*B. subtilis*) for production of surfactant compounds. The strains were cultivated for 7 days in different solid media and assessed for biosurfactant production using an inverted version of the drop-collapse assay with various oils. A key methodological innovation of this work was the development of a Python script to standardize and automate droplet diameter measurements. This approach minimized subjectivity, improved reproducibility, and enabled systematic data recording and comparative analysis across different culture conditions, providing an efficient and original tool for similar studies. Among the tested strains, two – BRB191, recovered from sediments of Itaguaré Beach, Bertioga – SP, and BRB592, isolated from sediments collected at Saco da Ribeira Beach, Ubatuba – SP), phylogenetically related to *B. mexicanus*, demonstrated promising results for biosurfactant production, however, the media used for bacterial growth did not impact tensoactive activity of bacterial cultures. Additionally, selected strains were subjected to media with varying salinity and pH to investigate potential effects on surfactant production. In such conditions, significant increase in surfactant activity was observed when strains we grown in higher salinity levels. Also, extracts from hit-strains were chemically analyzed to confirm the occurrence of surfactants. These findings underscore the biotechnological potential of marine *Bacillus* strains within the MicroMarin collection in the production of industrially valuable bioproducts. Furthermore, these results highlight the relevance of integrating computational tools for assay standardization and data analysis.

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