

**TITLE:** CHARACTERIZATION OF COMPLEX MIXTURE OF CYCLIC LIPOPEPTIDES PRODUCED BY *BACILLUS PARALICHENIFORMIS* ALONG THE GROWTH CURVE BY ESI-MS

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**ABSTRACT:**

Cyclic lipopeptides are a group of biosurfactants containing fatty acid and peptide chains that exhibit outstanding interfacial activities, which have many applications in agriculture, health, and particularly in the oil industry. The genus *Bacillus* is well known for producing various classes of lipopeptides such as surfactins, iturins, lichenysins, fengycins, plipastatin and polymyxins. They can be differentiated by their most striking biological activities like surface tension reduction, emulsification, de-emulsification, antifungal and/or antibacterial activities. The species of *Bacillus* generally synthesize a mixture of lipopeptides from different families generating a plethora of isoforms that differ in composition of the amino acids, length and branching of the fatty acids, by the chemical bond between the two domains and also in their biological properties. Therefore, analysis of the composition of isoforms may be a rapid and robust strategy to selected strains with surface activity tailored to desired activity. Hence, the aim of this study was to analyze the interfacial activities and composition of lipopeptides produced by a *Bacillus paralicheniformis* ICA58 along its growth curve. The bacterial strain was incubated in Mineral Medium (MM) containing glucose as carbon source, at 30 °C under 150 rpm for 48 h. Bacterial growth, surface tension and water/kerosene emulsification were monitored at different periods of time. The lipopeptides were purified by acid precipitation of cell-free culture broth. The lyophilized lipopeptides were dissolved in methanol and analyzed by Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer in the positive ion mode with internal calibration. The results showed that ICA58 secreted lipopeptides that reduced the surface tension to 27 mN/m at 16h of growth. After 24h the strain started to show emulsification activity that disappeared after 32h of cultivation, when was detected de-emulsify activity. The mass spectral analysis revealed that *B. paralicheniformis* ICA58 produces a complex mixture of lipopeptides analogous to lichenysin and polymyxin classes and a few unidentified compounds, probably involved with de-emulsification properties. In summary, it was noticed that the lipopeptide isoforms varied along the curve leading to differences in surface and interfacial activities, showing the lipopeptides composition can be guided by the time of cultivation and desired activity.

**Keywords:** *Bacillus paralicheniformis*, growth curve, lipopeptides, biosurfactant, mass spectrometry

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