

STRUCTURAL CHARACTERIZATION OF LIPOPEPTIDES PRODUCED BY *BACILLUS* MANGROVE-DERIVED STRAINS BY MALDI-TOF/TOF MS

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Lipopeptides are a group of biosurfactants containing fatty acid and peptide chains that exhibit outstanding biological- and interfacial-activities, which have many industrial applications. The aim of this study was to analyze the structure of lipopeptides produced by 5 *Bacillus* mangrove-derived strains (ICA13, TIM27, TIM49, TIM68 and TIM96) by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry. The lipopeptides were purified by acid precipitation of cell-free culture broth. The lyophilized lipopeptides were dissolved in methanol and distilled water (2 mg/mL) and one μL of this solution was spotted onto a AnchorChip target plate and allowed to air dry. Afterwards, the sample was covered with a equal volume of the matrix solution α -cyano-4-hydroxycinnamic acid. The monoisotopic masses were obtained in the positive ion reflector mode on the MALDI TOF/TOF MS UltrafleXtreme with external calibration. The mass spectral analysis revealed a complex mixture of the lipopeptides iturins, surfactins, and fengycins. It was detected two major clusters with peaks, varying the mass-to-charge ratio (m/z) between 1000 to 1100 and the other between m/z from 1400 to 1500. The first group of peaks is attributed to surfactin or iturin isoforms, while the second region is assigned to fengycin. It was observed that each peak is accompanied by a homologous series differing in m/z 14, corresponding to a methyl group. The main peaks detected in the strains ICA13, TIM68 and TIM96 were at m/z 1030, 1044, 1058, 1066, 1072, 1080, 1086, 1094, 1108 and 1121 corresponding to the protonated forms of iturins with fatty acid chains from C13 to C18. The strains TIM27, TIM49, TIM68, TIM96 exhibited surfactins A and B, with fatty acid chains having from C13 to C17. At least, 12 distinct masses (m/z 1008, 1017, 1022, 1031, 1036, 1045, 1059, 1060, 1073, 1074, 1089 and 1103) were identified as surfactin. All five strains exhibited fengycin isoforms containing fatty acid chains from C14 to C17. The TIM27, TIM49, TIM96 and ICA13 exhibited fengycin types A and B at m/z $[M+H]^+$ = 1436, 1450, 1464, 1478, 1492 and 1506; $[M+Na]^+$ = 1458, 1472, 1486, 1500, 1514, and 1528. TIM 68 exhibited only fengycin A at m/z $[M+H]^+$ = 1434, 1448 and 1462; $[M+Na]^+$ = 1456, 1470, 1484. The 5 strains analyzed exhibit at least two classes of lipopeptides, iturin and fengycin or surfactin and fengycin, this co-production by *Bacillus* spp. strains is indicative of its industrial potential.

Keywords: fengycin, surfactins, iturins, mass spectrometry

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