LIPOPEPTIDE PROFILING OF A *BACILLUS* STRAIN BY HPLC-HRMS TECHNIQUE

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Abstract

Surfactins, iturins and fengycins are lipopeptide-type biosurfactants produced by the gram-positive *Bacillus* strains. They consist of an oligopeptide amino acid loop and a hydrophobic fatty acid chain. These lipopeptides are proved to exhibit various biological activities, such as anti-tumor, anti-viral and anti-inflammatory effects. According to these properties, different therapeutic and environmental applications of these compound groups are considered. Their chemical composition may vary in the length of the fatty acid chain and in the sequence of the amino acids of the peptide loop, generating a wide spectrum of different homologues and isomers. These isoforms can be discerned by fragmentation in mass spectrometry. Depending on the cultivation conditions the production of biosurfactants is affected, resulting in various rates of the different isoforms produced.

In this work a mixture of lipopeptides were extracted from the *Bacillus* strain SZMC 6178J and was examined by HPLC-HESI-MS techniques. To increase the separation of the fractions with higher masses a gradient elution was applied using a non-polar solvent system, which led to the elution and characterization of their structures. The quantitative examinations of the effects of the culture media modified by various carbon sources and metal ions on the composition and ratio of the different surfactin isoforms produced were carried out by a triple quadrupole mass spectrometric system in full scan mode. We observed that the application of metal ions commonly used for enhancing surfactin production resulted in the almost complete loss of lipopeptide biosynthesis of the bacteria. The qualitative measurements of the carbon source modified samples were achieved by an OrbiTrap high resolution mass spectrometer in parallel reaction monitoring mode. Both the length of the linked fatty acids and the peptide sequences were also investigated during the MS^2 spectra analyses of the hydrogenated precursor ions. The results led to the detection and identification of 16 different surfactin variants. Observation of the relative ratios of the different homologues and isoforms showed that the [Sur] and [Val7] variants and molecules with C14 and C15 chain lengths were the most common in the samples.

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