CHARACTERIZATION OF NOVEL SURFACTIN ISOFORMS AND THE EFFECTS OF DIFFERENT CULTIVATION PARAMETERS ON THEIR PRODUCTION BY *BACILLUS SUBTILIS*

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Abstract

Surfactin is a lipopeptide-type biosurfactant produced mainly by the gram-positive microorganism *Bacillus subtilis*. It consists of a peptide loop of seven amino acids and a hydrophobic fatty acid chain (C_{12} – C_{16}). Surfactins 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 surfactins are considered. The chemical composition of surfactins could be varied in the length of the fatty acid chain and in the sequence of the amino acids of the peptide chain generating a wide spectrum of different homologues and isomers. The chemical composition of these isoforms could be elucidated via mass spectrometry by the analysis of MS^n fragmentation pattern. Furthermore, depending on the cultivation conditions, the production of surfactins are affected resulting in various rates of the different isoforms produced.

In this work a mixture of surfactins were extracted from the strain *Bacillus subtilis* (SZMC 6179J) and were examined by HPLC-ESI-IT-MS technique. To increase the separation of the components with higher masses a gradient elution was applied using a non-polar solvent system, which led to their proper elution and characterization of their structures. Both the length of the linked fatty acids and the peptide sequences were also investigated during the MS^2 spectra analyses of the sodiated precursor ions. The results led to the discovery of a novel, recently unknown group of surfactin forms possessing glutamic acid in as the fifth amino acid residue instead of the aspartic acid described previously at this position. To examine the effects of the culture media on the surfactin production, it was modified with various carbon sources and metal ions. It was reaffirmed that altering the cultivation parameters could enable the improved production of certain surfactin variants, which could serve possibility for their further preparative purification and structural elucidation.

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