Cytoplasmic membrane adaptation to surfactin in Bacillus subtilis 168 non-producing strain

Surfactin, the most potent surface active compound and antibiotic is produced by bacteria of the genus Bacillus. Surfactin interacts with membrane bilayers, that results in destabilization and permeabilization of this structure. However mechanism of surfactin self-resistance in the producer's membrane is not understood. The aim of this study was to characterize the adaptive processes occurring at the level of cytoplasmic membrane of surfactin non-producing strain B. subtilis 168, which was exposed to exogenously added surfactin during the exponential phase of growth. The cultivation protocol of B. subtilis growth on agar media plates supplemented with surfactin was developed. Two surfactin concentrations that inhibit (400 g/ml) and even stimulate (300 g/ml) the growth of B. subtilis 168 strain were assessed. Surfactin brought about the growth arrest for 3 hours and the restored growth rate decreased in the case of inhibitory concentration, whereas the stimulatory concentration increased the growth rate and resulted in higher final density of the population. TLC was performed to analyze the polar head groups of membrane phospholipids. The portion of phosphatidylserine was found to increase at both surfactin concentrations. The change of the membrane surface was accompanied with the substantial reconstruction of membrane fatty acids as revealed by GC/MS. The content of 16:0 and 18:0 fatty acids considerably increased whereas the non-branched fatty acids decreased. The resulting rigidization of the membrane interior was confirmed by the steady-state fluorescence anisotropy of DPH. In the membrane proteom of bacteria inhibited by surfactin was detected a new protein, that was identified by MS-MALDI as manganese-binding lipoprotein MntA. The obtained data outline an extensive reconstruction of cytoplasmic membrane in response to surfactin and contribute to the understanding of the membrane tolerance strategy.

Key words: Bacillus subtilis, surfactin, cytoplasmic membrane, resistance