ABSTRACT

Surfactin, a secondary metabolite produced by Bacillus subtilis, is a surface active compound and antibiotic permeabilizing membrane bilayer. The aim of this study was to reveal the self-resistance strategy at the level of the lipid moiety of cytoplasmic membrane, which B. subtilis employs to combat surfactin in concentrations that are lethal for other bacterial species. Non-producing strain B. subtilis 168 was cultivated in the presence of two different sublethal concentrations of surfactin (350 a 650 µg/ml), which was isolated from the culture broth of B. subtilis ATCC 21332. Presence of surfactin in the medium resulted in a concentration dependent lag phase, which took 40 min (350 µg/ml) and 3 h (650 µg/ml), respectively. Afterwards, the culture grew with the altered doubling time of 44 min (350 µg/ml) and 126 min (650 µg/ml), respectively. Surfactin induced substantial changes in the phospholipid composition of the cytoplasmic membrane. The proportion of the major phospholipid component phosphatidylglycerol decreased and inversely, the level of phosphatidylethanolamine increased. Interestingly, the content of phosphatidic acid rose considerably in the presence of surfactin concentration causing stimulation of B. subtilis growth (350 µg/ml). Liposome leakage assay using phospholipids mimicking the B. subtilis membrane adapted to surfactin showed that the higher proportion of phosphatidylethanolamine attenuates the permeabilizing effect of surfactin on the membrane. The fatty acid analysis revealed that the presence of surfactin in the concentration of 350 µg/ml led to decrease in the proportion of branched-chain fatty acids. Steady state fluorescence anisotropy of DPH labelled membrane lipids confirmed the higher rigidity in the aliphatic chain region. We concluded that the observed adaptive modifications might provide self-tolerance to the membrane active surfactin.

Key words:
surfactin, Bacillus subtilis, cytoplasmic membrane, phospholipids, fluidity