Biological and technical variability of Cronobacter sakazakii protein identification method

P-02.5-27

J. Novotný[I], A. Jelínková[I], B. Svobodová[I], L. Karamonová[I]

[I]Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 3, 166 28, Prague 6, Prague, Czech Republic

Cronobacter spp. are opportunistic food-borne pathogens. These Gram-negative bacteria could cause rare but life-threatening infections such as meningitis, necrotizing enterocolitis and sepsis, especially to immunosuppressed individuals and newborns with low birth weight. According to epidemiological studies, *Cronobacter*-related infections are caused mainly by specific sequence types (ST) within *Cronobacter* genus. *Cronobacter sakazakii* (ST4) used in this study is often associated with serious neonatal meningitis. Although several virulence factors have been described, the process of *Cronobacter* pathogenesis remains unclear and it would be useful to find a reliable method that enables to identify as many bacterial proteins as possible. In this study, we used the subfractionation method for isolation of outer membrane, inner membrane, periplasm and cytosol of *Cronobacter sakazakii* (ST4). Proteins of these fractions were purified with SDS-PAGE, digested with trypsin and then identified by mass spectrometry coupled with liquid chromatography (LC-ESI-Q-TOF MS). Essential steps of analysis including subfractionation, sample preparation, trypsin in gel digest and protein identification by mass spectrometry were performed three times. The results show biological and technical variability of this method. In total, more than 1300 proteins were identified, which is approximately 33 % of the predicted *Cronobacter sakazakii* proteom.