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Quantification of microbial genomic DNA by capillary electrophoresis and identification of phosphorus impurities

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There are many analysis techniques for DNA quantitation – measurement of UV absorbance(intact genomic DNA and hydrolytic genomic DNA), intercalation of fluorescence dyes(PicoGreen), quantitation of phosphorus by inductively coupled plasma optical emission spectrometry(ICP-OES), and dNMPquantitaion by capillary electrophoresis(CE) using dNMPcertified reference material(CRM) produced from Korea Research Institute of Science and Standard, KRISS. We purified genomic DNA from E. coli and B. subtilis with three different methods-the organic solvent extraction with cetyltrimethyl ammonium bromide(CTAB), ion exchange chromatography using anion exchange column, and silica gel adsorption. Quantity of each microbial genomic DNA was determined by several different methods as mentioned above. Interestingly, genomic DNA from B. subtilis was analysed that it contains large amount of phosphorus by ICP-OES compared with E. coli, a gram negative bacterium. The main two differences between the gram positive bacteria and the gram negative bacteria are cell wall thickness and composition of peptidoglycan layer. In the gram positive bacteria cell wall, peptidoglycan layers are anchored by wall teichoic acids(WTAs) which are covalently attached and extend through the cell wall and lipoteichoic acids(LTAs) which are polymers containing an alditol phosphate in their repeating units(glycerol phosphate or ribitol phosphate). Both types of teichoic acids contain phosphate groups and anion charged polymers. For this reason, WTAs and LTAs were assumed as genomic DNA quantitation interfering molecules. ICP-OES has been regarded as the most accurateand traceablemethod for quantitation of oligonucleotide, plasmid and human genomic DNA. Based on our study, dNMP quantitation by CE is more accurate method for quantitation of gram positive bacterial genomic DNA than ICP-OES.Moreover, we confirmed that phosphorus contaminants are sort of teichoic acid by RP-HPLC, LC-ESI-MS/MS, NMR.

Biography

H.-J.Yang got her master's degree at the age of 28 years from Yonsei University. She has studied at the University of Science and Technology, UST in Korea as a Ph.D student since 2009. Her major is bioanalysis science and she makes a study about methodswhich are quantification of genomic DNA and certification of reference material.

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