P116. Improved Method For The Extraction Of Humic Acid-Free DNA From Lignocellulosic Residues For Metagenomic Studies

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The isolation of a high molecular weight DNA is essential for successful metagenomic studies aiming to screen and exploit the variety of microorganisms inhabiting an environment [1]. In lignocellulosic rich samples is common to find contaminants like humic substances that are formed by the decomposition of plant, animal and microbial biomass [2]. In this sense, four DNA extraction methods were evaluated with respect to the quality and purity of DNA extracted from samples collected in a composting unit which handle lignocellulosic residues. In the methods A, B and C, the same chemical and enzymatic cell lysis and purification protocol was used, differing only in the type of humic substance removal agent (HSRA) added: (A) no agent [3]; (B) Cetyltrimethylammonium bromide (CTAB) 1% + β-mercaptoethanol 0,2%; (C) CTAB 1% + Polyvinylpyrrolidone (PVP) 1%. In method D, sodium phosphate was added to keep the DNA integrity, and CTAB 1% + $CaCl_2$ were applied as HSRA [4]. Furthermore, chloroform/isoamyl alcohol (24:1) and isopropanol were used in the purification step. Humic acid was extracted from the initial composting sample by acid precipitation [5] and quantified, as well as the humic acid content in DNA solution, by absorbance measurements at 340 nm. Metagenomic DNA of good quality was efficiently isolated from the composting sample using the method D (54.59 µg/g of compost). The average DNA yield obtained with the other methods was only 7.80 µg/g of compost. An assessment of the extracted DNA purity demonstrated that method D provided the purest DNA with an absorbance ratio $A_{260/280}$ of 1.86 and $A_{260/230}$ of 1.80. DNA solution obtained from the method B presented the highest humic acid content (1.482 µg/g), thus justifying the low absorbance ratio $A_{260/230}$ observed (0.86), and highlighting consequently the low efficiency of this method in the humic acid removal (46.29%). The method D ensured higher yield of good quality contamination-free DNA in comparison to other methods evaluated in this study. The addition of $CaCl_2$ allowed the binding of humic substances to the calcium ions which greatly contributed to eliminate humic impurities prior to cell lysis.