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Extracellular DNA in soils: quantitative assessment, binding to soil and resistance to degradation

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It is generally assumed that nucleic acids (DNA and RNA) can be released into environment only after cell lysis. However recently it was found that nucleic acids could be 'purposely' excreted by metabolically active cells to form a organized network of extracellular DNA, eDNA [1]. There are some indications that eDNA participates in transformation reactions *in situ* and catalyzes development of biofilms[3]. Extracellular DNA in marine sediments was found to exceed the content of intracellular DNA by 10-40 times [2]. The present communication deals with eDNA in soils including development of analytical techniques and degradation studies.

DNA was extracted by two methods: 1) popular procedure [4] based on cell lysis with proteinase K and SDS and DNA extraction with PBS-STAB buffer (pH 8) under heating, and 2) by alkaline extraction (0.5 N NaOH, 55^{o}C, 24 h) of soil with following DNA separation, hydrolysis and HPLC analysis of released nitrogen bases. Contrary to [2] we failed to find any significant amount of DNA without cell lysis, therefore method 1 was assumed to represent intracellular DNA. Method 2 gave essentially higher DNA yield. From the base composition (T+C$>$A+G), we concluded that NaOH-extracted DNA was partly apurinated.

To assess an amount of eDNA, soil samples were incubated with added substrates to induce intensive microbial growth. The development of bacteria, fungi and protozoa was followed by direct microscopy and FAME profiling. The plot of DNA (y) versus microbial biomass (x) was approximated by linear regression (y=a+bx) with two parameters: slope b was the intracellular DNA content per cell mass unit and intercept a corresponded to eDNA. It is interesting that plot of DNA extracted by method 1 passed through the origin (the intercept a was close to zero), while the second method

produced DNA plot with similar slope (b) and a much higher a-value. Therefore, a relatively mild DNA isolation technique based on cell lysis at pH 8.0 selectively extracts only the intracellular DNA, while drastic alkaline treatment releases both intracellular and extracellular DNA.

It was shown that decomposition of eDNA and eRNA in soils followed the first-order kinetics. The effects of environmental factors (temperature, moisture, pH, ...) was similar to other biodegradation processes. The unique for nucleic acids retardation factor was their binding to Fe-saturated bentonite, goethite, gibbsite and amorphous Fe-/Al-hydroxides. The adsorption of eDNA and eRNA was mostly irreversible and resulted in dramatic slowdown of biodegradation. The highest content of eDNA was found in ferralitic soils characterized by high content of such minerals.

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