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Thermochemolysis with trimethylsulfonium hydroxide in fatty acid analysis: a tool for biochemical investigations in enchytraeids

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Enchytraeids are distributed globally and are common in most soils, in which they play important roles in food web and organic matter decomposition. Fatty acids are ubiquitous constituents of all organisms, and are important as energy storage and membrane components. Fatty acid analyses in tissues are an important tool to understand the physiological status of a species, its trophic behaviour/diets, microbial symbioses, and chemosystematics. Tissue analysis typically involves a solvent extraction followed by base hydrolysis and methylation of acid groups (FAME). However, the reduced size of enchytraeids limits the use of conventional methods which would demand considerable amount of sample material, time, and have higher risk of contamination. Thermochemolysis with trimethylsulfonium hydroxide (TMSH) using pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) results in on-line methylating of samples that are readily separated and detected. This analytical technique has been applied to the analysis of fatty acid profiling of various types of samples. Therefore, the aim of this study was to evaluate the practical use of this technique with enchytraeids. Adults of *Enchytraeus crypticus* were separated from cultures maintained in natural soil and fed weekly with oatmeal flakes. They were briefly washed and transferred to agar substrate and after 14 days, healthy adult individuals were cleaned in deionized water for 24 h, treated individually with TMSH, and had their fatty acid profile determined by Py-GC-MS. The method was sensitive to detect the fatty acid composition of individuals. A total of 10 fatty acids were detected in enchytraeid tissues in the range C10:0 to C16:0. A remarkable percentage of C14 acids was present: tetradecanoic acid (C14:0) and the unsaturated ω 5 tetradecenoic acids (C14:1 ω 5) were the most abundant compounds. (Z)-7 and (Z)-9 tetradecenoic and hexadecenoic acids were also detected, albeit in lower abundance. The fatty acid distribution obtained by TMSH is consistent with the fatty acid distribution reported in the only paper found so far describing a conventional method used for enchytraeids. In conclusion, TMSH reaction using Py-GC-MS is an interesting approach in fatty acid analysis of enchytraeids. The usefulness of this technique to compare different enchytraeid species reared in different conditions is currently under investigation.