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POLAR PYROLYSIS PRODUCTS FOR A SENSITIVE FINGERPRINT CHARACTERIZATION OF ORGANISMS BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

Pyrolysis gas–liquid chromatographic and pyrolysis mass spectrometric techniques applied to biopolymers are normally based on the more volatile compounds derived from pyrolysis degradation. It is demonstrated that the less volatile and more polar compounds are key components of pyrolyzates based on smaller organisms. After silylation, these compounds can easily be analyzed by gas–liquid chromatographic techniques, resulting in a sensitive fingerprinting characterization for whole organisms.

INTRODUCTION

The characterization of microorganisms or tissue samples by pyrolysis gas–liquid chromatography (GLC) has been established as a reliable method providing a characteristic fingerprint for the substrate under investigation. Pyrolysis GLC data obtained under controlled experimental conditions have been shown to be adequate for the identification of closely related strains of bacteria[1], of potential pathogens[2] and for the diagnosis of fungal and viral diseases in plants[3]. These fingerprints consist of characteristic patterns of GLC peaks whose molecular composition need not be known in order to differentiate one pyrolyzed substrate from another. Additional information on the original composition of the substrate can be obtained by the mass spectrometric identification of pure compounds in the GLC eluate[1,4]. The method can be modified by coupling a pyrolyzer directly to a mass spectrometer. The pattern of fragments thus obtained is a unique feature of a given matrix and can be used for identification purposes[5–7].

So far, the pyrolytic characterization of biological substrates has been based mainly on the less polar and more volatile components of their pyrolyzates. An extension of GLC analysis towards less volatile compounds would thus increase the amount of available information and could help in obtaining a better understanding of the chemistry of pyrolysis processes. Preliminary experiments are described in which less volatile components of pyrolyzates are used for the GLC fingerprinting of different organisms.

MATERIALS AND METHODS

Microscopic organisms were removed from sea water and culture media by filtration through Whatman GF/C glass-fibre filters, which are thought to be adequate for the collection of planktonic algae and large bacteria. Before use, the filters were ignited at *ca.* 500° for at least 12 h in order to remove trace organic contaminants.

The organisms used were bacteria (*Escherichia coli*, strain K12, grown on a Zobell medium), algae (*Chlorella* sp., strain H57/3-20-72, and *Navicula* sp., strain Q39/5-10-72), both grown on an Erdschreiber medium, and a natural mixed plankton population taken from Kiel harbour on April 24th, 1974. After filtration, the filters with the collected organisms were washed with particle-free sea water. The filters were dried at 150° for 2 h and stored in a deep-freezer below −18°.

For pyrolysis, the filters were cut into narrow strips with an average length of 2 cm, which carried between 6 and 30 μg of protein (determined with Folin reagent[8]). The strips were placed into a Pyrex glass tube with an O.D. of 2.3 mm to fit the pyrolyzer and the glass tube containing the sample was dried for 30 min at 150°. Through a puncture in a silicon rubber septum, the open end of the glass tube was then quickly connected to a helium container under atmospheric pressure. In order to guarantee a good gas exchange, the empty end of the glass tube was heated twice over a gas flame, with a sufficient time interval between each heating period to allow for cooling. Immediately after a third heating, the tube was sealed near the septum. The sample was thus enclosed under an estimated helium pressure of *ca.* 400 torr.

The samples were pyrolyzed with a Pyroprobe 150 (Chemical Data Systems, Oxford, Pa., U.S.A.) or by direct heating of the sealed glass tubes in a gas flame. In the Pyroprobe, the loaded part of the glass tube was placed into the heating coil, the casing of which was maintained at 200°. One third of the tube protruded from the casing, and the temperature of this part of the glass tube was only slightly elevated. After an adaptation period of 2 min, the coil was heated at the rate of 20°/msec to a temperature limit of 500°. Alternatively, the pyrolysis was performed by moving the loaded part of the sealed glass tube through the tip of a gas flame for *ca.* 1 sec. In both instances the pyrolysis products condensed in the cooler part of the tube. Their compositions were found to be similar regardless of the method employed.

After pyrolysis, the glass tubes were opened and the filter strips removed. For further analysis, the pyrolyzate was used either untreated in pentane solution or after silylation with N-methyl-N-silyltrifluoroacetamide (MSTFA)[9]. For silylation we added 10 or 20 μl of re-distilled MSTFA (Macherey, Nagel & Co., Düren, G.F.R.) containing catalytic concentrations of trimethylchlorosilane. The tubes were then re-sealed and maintained at 120° for 30 min.

GLC analyses were carried out with a Varian Model 2700 gas chromatograph with a flame ionization detector, and 2-m × 3-mm I.D. glass columns packed with Chromsorb W, AW-DMCS, 60–80 mesh (Macherey, Nagel & Co.), coated with 5% OV-101 (ref. 10). The injection port and detector temperatures were 320°, while the column temperature was programmed from 80 or 100° to 320° at 10°/min. Helium was used as carrier gas at the flow-rate of 40 ml/min. IR spectra were measured with a Beckman IR-33 spectrometer equipped with an attenuated total reflection (ATR) adapter (Harrick Scientific Corp., Ossining, N.Y., U.S.A.).

RESULTS AND DISCUSSION

Pyrolysis products from biopolymers usually contain hetero-atoms such as oxygen, nitrogen and, in some instances, even sulphur. It seems to be justified to assume that pyrolysis products reflect the elemental composition of the pyrolyzed substrate. On closer inspection, however, it is found that the O:C and N:C elemental ratios of pyrolyzates reported to date are below those known for the substrates. The average O:C and N:C ratios of pyrolyzates of dried bacteria can be calculated from the elemental composition and the relative abundance of their main components derived from GLC and mass spectrometric data, and were found to be of the order of 1:9 and 1:15 (ref. 1). The O:C and N:C ratios of dried bacteria, however, are closer to 1:4 and 1:7, respectively (derived from tables for the elemental composition of organisms[11]). This indicates that in these pyrolyzates, polar compounds with a relatively high content of hetero-atoms have, so far, escaped detection. It is thought that these substances might be of considerable aid in the identification and characterization of tissues and microorganisms based on pyrolytic fingerprinting.

Preliminary tests with pyrolysis products from different organisms confirmed the polar character of many of these compounds. The pyrolyzates were only partly soluble in *n*-pentane or benzene; diethyl ether or chloroform dissolved a larger proportion, and methanol or trifluoroacetic acid left only trace amounts undissolved.

Fig. 1 shows a characteristic IR spectrum. The broad band centred around 3300 cm^{-1} for OH- and NH- valence vibrations is very intense relative to the CH- band near 2900 cm^{-1}. A group of strong bands between 1600 and 1750 cm^{-1} may be attributed to saturated and unsaturated carbonyl compounds, C=N double bonds in heterocycles, amides, etc. Two weak band areas between 1460 and 1350 cm^{-1} and between 1230 and 1150 cm^{-1} indicate the presence of polar sulphur bonds such as $-SO_2-O-$. Pyrolyzates of different organisms gave rise to very similar spectra, confirming the general validity of our assumptions.

An attempt to separate untreated pyrolyzates on the OV-101 GLC column resulted in rather featureless gas chromatograms. However, after silylation, characteristic GLC fingerprints were obtained from different algae. Fig. 2 shows the gas chromatogram of the pyrolyzate of a *Chlorella* sp. An amount equivalent to 19 μg of protein was pyrolyzed and one fifth of the pyrolyzate dissolved in 2 μl of MSTFA

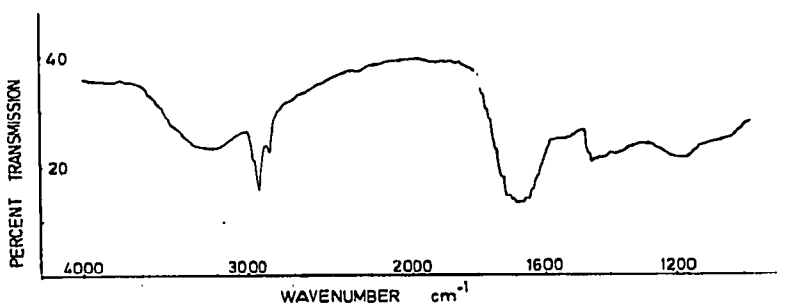

Fig. 1. Typical IR spectrum of pyrolyzates of a mixed plankton population.

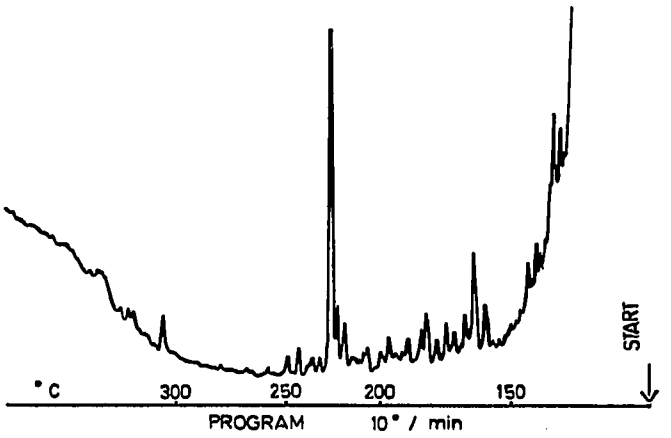

Fig. 2. Chromatogram from a temperature-programmed GLC run for silylated pyrolysis products derived from a *Chlorella* sp.; temperature programme started at 80°.

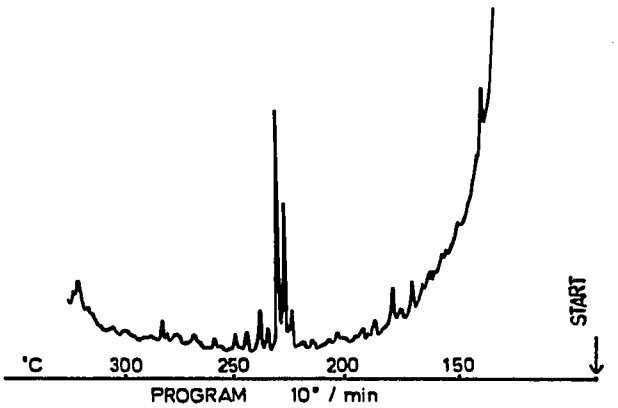

Fig. 3. Chromatogram from silylated pyrolysis products from a *Navicula* sp.

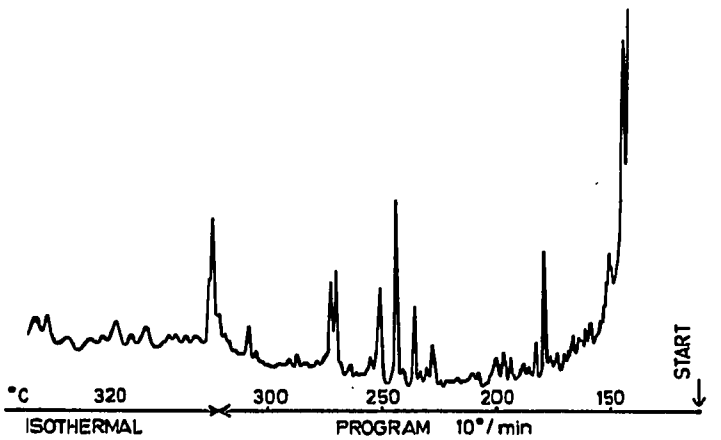

Fig. 4. Chromatogram from silylated pyrolysis products from a mixed natural plankton population; temperature programme started at 100°.

was injected on to the column. Fig. 3 depicts the pyrolysis gas chromatogram of a *Navicula* sp. The pattern produced by a mixed natural plankton population consisting mainly of *Skeletonema costatum* (Grev.) and of small crustaceae such as copepods in various stages of development is shown in Fig. 4.

Summarizing the results, confirmation has been obtained of our assumption that pyrolysis compounds based on biopolymers such as smaller organisms contain a relatively high content of hetero-atoms. Thus, IR spectra indicate a substantial contribution of polar substances to pyrolyzates. For sensitive GLC analysis, derivatization is required in order to increase the volatility of these polar non-volatile compounds, resulting in characteristic fingerprinting. This might aid considerably in the GLC identification of tissues and microorganisms based on pyrolysis and it might help to obtain a better understanding of pyrolysis processes themselves.

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REFERENCES

1 P. G. Simmonds, *Appl. Microbiol.*, 20 (1970) 567.
2 E. Reiner and W. H. Ewing, *Nature (London)*, 217 (1968) 191.
3 A. Myers and L. Watson, *Nature (London)*, 223 (1969) 964.
4 H. R. Schulten, H. D. Beckey, H. L. C. Meuzelaar and A. J. H. Boerboom, *Anal. Chem.*, 45 (1973) 191.
5 H. L. C. Meuzelaar and P. G. Kistemaker, *Anal. Chem.*, 45 (1973) 587.
6 H. L. C. Meuzelaar, M. A. Posthumus, P. G. Kistemaker and J. Kistemaker, *Anal. Chem.*, 45 (1973) 1546.
7 H. L. C. Meuzelaar, M. A. Posthumus, P. G. Kistemaker and J. Kistemaker, *Anal. Chem.*, 45 (1973) 2358.
8 O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
9 M. Donike, *J. Chromatogr.*, 42 (1969) 103.
10 M. Donike, *Chromatographia*, 6 (1973) 190.
11 J. R. Geigy, *Wissenschaftliche Tabellen*, 1968, 798 pp.