CHARACTERISATION OF PATHOGENIC SPECIES OF *CANDIDA* BY GAS CHROMATOGRAPHY: PRELIMINARY FINDINGS

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SUMMARY. The volatile substances produced and utilised by some species of *Candida* isolated from man, were determined in two cultural conditions by gas chromatography. It was possible to characterise *Candida krusei*, *C. parapsilosis*, *C. tropicalis*, *C. albicans* and *C. guilliermondii*, but more species and isolates would have to be studied for conclusive results.

INTRODUCTION

The concept of identification of bacteria, especially anaerobes, by the detection of their metabolites by gas chromatography, has been described by several authors, e.g., MacGee (1968), Mitruka and Alexander (1968), Rogosa and Love (1968), Henkel (1971), Carlsson (1973), Wade and Mandle (1974), Mayhew and Gorbach (1977), van Assche (1978), Lategan, du Preez and Potgieter (1978). Papers on the classification and characterisation of bacteria by gas chromatography have been published, but we have been unable to find references, in this connection, to the pathogenic yeasts. This report describes the metabolites produced by *Candida* in screw-capped bottles and in shake-flask cultures and detected by a gas-chromatographic procedure that allowed the direct injection of culture supernates (fluids).

MATERIALS AND METHODS

Strains of *Candida*. Two isolates of each of the following yeasts, from human clinical specimens, were obtained from the Department of Medical Microbiology, University of Pretoria: *C. albicans* (one each of type A and type B), *C. tropicalis*, *C. krusei*, *C. guilliermondii* and *C. parapsilosis*.

Culture media. The organisms were cultivated in duplicate in Sabouraud's broth, containing Peptone (E. Merck, Darmstadt, W. Germany) 10 g, D-glucose anhydrous (E. Merck) 20 g, distilled water 1 L.

Ten ml of the medium in 25-ml screw-capped bottles and 50 ml in 250-ml Erlenmeyer flasks with cotton plugs were inoculated with 0.1 ml and 0.5 ml respectively of 24-h culture and incubated at 30°C for 48 h before analysis by gas chromatography. Uninoculated media were incubated as controls.

Gas-chromatographic analyses. After incubation the cells were centrifuged at 3000 *g* for 30 min, and 1 ml of 20% (v/v) formic acid (E. Merck) was added to 4 ml of supernate to minimise ghosting (du Preez and Lategan 1976, 1978). α -Picoline (2-methylpyridine) (British Drug Houses, Fane Road, Poole, Dorset) was added as an internal standard. A Varian 800 autosampler (Varian Inc., Palo Alto, Calif, USA) was used for the on-column injection of 3- μ l sample volumes into a 5830A gas chromatograph (Hewlett Packard Avondale, Penn, USA) with an FID detector and electronic integrator. Between each determination the column was purged twice with 3- μ l injections of 20% formic acid.

Porapak N (Waters Assoc., Mass, USA) packed in a 1.5 $\times$ 4-mm internal diameter glass column was used.

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Operating temperatures were: column 185°C, inlet 220°C, detector (FID) 240°C. The carrier gas was nitrogen at 50 ml min⁻¹.

Some of the metabolites were identified by retention times on the column by comparison with those of the pure chemicals, and confirmed on a column of Carbowax 20 M (Analabs Inc., North Haven, Conn, USA). The peak areas of the control media were subtracted from the values obtained in the culture media.

The analyses showed that the Sabouraud's medium used for cultivation contained acetic acid, propionic acid, valeric acid, isovaleric acid and two unidentified volatile substances with retention times of 6.83 min and 20.8 min respectively. A peak with a retention time of 4.5 min appeared and valeric acid disappeared in the shake flasks containing the uninoculated medium. Some of these compounds were utilised by the yeast and the utilisation was expressed as a percentage of the original concentration in the medium. Acetic acid and isovaleric acid were also produced by some of the candida species and the peak areas, after subtraction of the peak areas in the control medium, are presented.

All data were corrected according to the mean of the values obtained with the internal standard.

RESULTS AND DISCUSSION

The results obtained with the organisms in closed bottles are presented in table I and for the shake cultures in table II.

Ethanol was produced by all the species and, as expected, more was produced in the closed bottles. *C. krusei* was easily recognised by the production of an unidentified metabolite with a retention time of 3.77 min in both conditions. It was also the only organism that produced acetic acid in aerobic conditions. *C. parapsilosis* was the only organism that produced acetic acid in the closed bottles. *C. tropicalis* utilised less isovaleric acid than the other species (table I). The two remaining species, *C. albicans* and *C. guilliermondii*, could be separated because in the capped bottles the former produced less ethanol (table I) and in the shake flasks approximately three times more isovaleric acid (table II).

Acetoin was produced by all the candida species but more was produced in the aerobic conditions. This is in accordance with our experience with genera of the Enterobacteriaceae

TABLE I

Production of metabolites and percentage removal of volatile substances from the medium by candida species in screw-capped bottles

Species	Production (+ values*) or removal (– values†) of indicated substance at retention time (min) of								
	1.23 Ethanol	3.77 Unident- ified	5.2 Acetic acid	6.83 Unident- ified	8.59 Propionic acid	13.15 Acetoin	20.8 Unident- ified	27.5 Isovaleric acid	33.0 Valeric acid
<i>C. albicans</i> type A	+4550	...	–72%	–50%	–85%	+17	–84%	–100%	–88%
<i>C. albicans</i> type B	+3697	...	–81%	–60%	–79%	+13	–82%	–100%	–69%
<i>C. tropicalis</i>	+4281	...	–76%	–90%	–100%	+32	–100%	–2.5%	–100%
<i>C. tropicalis</i>	+6438	...	–83%	–90%	–100%	+22	–100%	–8%	–100%
<i>C. krusei</i>	+2145	+28	–40%	–17%	–44%	+16	–50%	–42%	–63%
<i>C. krusei</i>	+1779	+25	–52%	–10%	–43%	+13	–42%	–58%	–32%
<i>C. guilliermondii</i>	+6294	...	–78%	–91%	–100%	+18	–100%	–58%	–100%
<i>C. guilliermondii</i>	+6019	...	–86%	–92%	–100%	+20	–95%	–25%	–100%
<i>C. parapsilosis</i>	+2124	...	+24	–44%	–100%	+15	–100%	–100%	–100%
<i>C. parapsilosis</i>	+2418	...	+44	–18%	–100%	+18	–100%	–100%	–100%

* Peak area (10⁻³).

† Percentage of original concentration in culture medium.

TABLE II

Production of metabolites and percentage removal of volatile substances from the medium by Candida species in shake flasks

Species	Production (+ values*) or removal (– values†) of indicated substance at retention time (min) of								
	1·23 Ethanol	3·77 Unident- ified	4·5 Unident- ified	5·2 Acetic acid	6·83 Unident- ified	8·59 Propionic acid	13·15 Acetoin	20·8 Unident- ified	27·5 Isovaleric acid
<i>C. albicans</i> type A	+32·5	...	–100%	–61·5%	–80%	–100%	+106	–100%	+66
<i>C. albicans</i> type B	+1052	...	–100%	–57·5%	–99%	–100%	+212·5%	–92%	+84
<i>C. tropicalis</i>	+464	...	–100%	–14%	–94%	–91%	+171·5	–100%	+73
<i>C. tropicalis</i>	+354	...	–100%	–52%	–93%	–91%	+174·5	–100%	+72
<i>C. krusei</i>	+785·5	+49	–100%	+68	–81%	–100%	+104	–100%	+V
<i>C. krusei</i>	+340	+40	–100%	+54·4	–83%	–100%	+101	–100%	+V
<i>C. guilliermondii</i>	+507	...	–100%	–0%	–92%	–89%	+151	–97%	+23
<i>C. guilliermondii</i>	+566·5	...	–100%	–19%	–93%	–88%	+148	–95%	+17
<i>C. parapsilosis</i>	+1156	...	–100%	–21·4%	–78·1%	–100%	+92	–100%	+64
<i>C. parapsilosis</i>	+651	...	–100%	–42·9%	–78·5%	–100%	+80	–100%	+49

V = variable.

* and † As in table I.

(van Vuuren, Lategan, Erasmus, unpublished data) and *Staphylococcus aureus* (Lategan *et al.*, 1978).

In these experiments it was possible to characterise *C. krusei*, *C. parapsilosis* and *C. tropicalis*, *C. albicans* and *C. guilliermondii*. For a conclusive indication on the value of gas chromatography in identifying pathogenic yeasts, a comprehensive study on more species and more representative strains is necessary. The present study suggests that this deserves investigation.

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REFERENCES

- CARLSSON, J. 1973. Simplified gas chromatographic procedure for identification of bacterial metabolic products. *Appl. Microbiol.*, **25**, 287.
- DU PREEZ, J. C. AND LATEGAN, P. M. 1976. Gas chromatographic determination of C₂-C₅ fatty acids in aqueous media with a Porapak N column. *J. Chromatogr.*, **124**, 63.
- DU PREEZ, J. C. AND LATEGAN, P. M. 1978. Gas chromatographic analysis of C₂-C₅ fatty acids in aqueous media using Carbopack B-Carbowax 20M-phosphoric acid. *J. Chromatogr.*, **150**, 259.
- HENKEL, H. G. 1971. Gas chromatographic analysis of low boiling fatty acids in biological media. *J. Chromatogr.*, **58**, 201.
- LATEGAN, P. M., DU PREEZ, J. C. AND POTGIETER, H. J. 1978. Preliminary findings on the characterization of bacteria causing mastitis by gas chromatography. *Br. vet. J.*, **134**, 342.
- MACGEE, J. 1968. Characterization of mammalian tissues and microorganisms by gas chromatography. *J. gas Chromatogr.*, **6**, 48.
- MAYHEW, J. W. AND GORBACH, S. L. 1977. Internal standards for gas chromatographic analysis of metabolic end products from anaerobic bacteria. *Appl. envir. Microbiol.*, **33**, 1002.
- MITRUKA, B. M. AND ALEXANDER, M. 1968. Rapid and sensitive detection of bacteria by gas chromatography. *Appl. Microbiol.*, **16**, 636.
- ROGOSA, M. AND LOVE, L. L. 1968. Direct quantitative gas chromatographic separation of

C₂-C₅ fatty acids methanol and ethyl alcohol in aqueous microbial fermentation media. *Appl. Microbiol.*, **16**, 285.

VAN ASSCHE, P. F. D. 1978. Differentiation of *Bacteriodes fragilis* species by gas chromatographic detection of phenylacetic acid. *J. clin. Microbiol.*, **8**, 614.

WADE, T. J. AND MANDLE, R. J. 1974. New gas chromatographic characterization procedure: preliminary studies on some *Pseudomonas* species. *Appl. Microbiol.*, **27**, 303.