

Characteristic γ -Lactone Odor Production of the Genus *Pityrosporum*

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Mass spectrometric-gas chromatographic analysis of culture headspaces revealed that members of the genus *Pityrosporum* produce volatile γ -lactones during growth on lipid-containing media. Representative members of other yeast genera found on humans failed to produce these compounds. Addition of lecithin, oleic acid, triolein, or human sebum to the culture media stimulated γ -lactone production by *Pityrosporum* species. All yeasts tested produced isopentanol and phenylethanol. Production of γ -lactones may serve as a valuable characteristic in the identification of organisms of the genus *Pityrosporum*.

The genus *Pityrosporum* is comprised of four species, *P. ovale*, *P. orbiculare*, *P. canis*, and *P. pachydermatis* (5). The first two are found on humans in areas rich in sebaceous glands such as the face and trunk, and *P. ovale* is the most frequent organism found on the scalp (8). Despite their widespread distribution, members of this genus are ill defined, and morphological characteristics serve as the prime method of identification (5). In 1964, van Abbe noted that *P. ovale* emits a distinctive, fruity odor when cultured on Sabouraud agar overlaid with olive oil (11). We have similarly noticed a distinctive fruity, "canned-peach" odor produced by *P. ovale* and became interested in the odor because of its potential use in the identification of *Pityrosporum* species and its possible contribution to human scalp odors.

In this study, the volatiles produced by the genus *Pityrosporum* and other yeast genera were collected and concentrated from the headspace of the culture. Gas chromatography (GC)-mass spectrometry was used for the separation and structure elucidation of the volatiles.

MATERIALS AND METHODS

Microorganisms and culture media. *P. ovale* strains ATCC 24047, 12078, and 14521, *P. pachydermatis* ATCC 24022, *P. canis* ATCC 14522, *P. orbiculare* ISG 6331 (obtained from M. Nazarro-Porro, Institute of Dermatology, St. Gallicano, Rome, Italy), *Candida albicans*, *Saccharomyces cerevisiae*, *Rhodotorula rubra*, and *Torulopsis glabrata* (Duhring Laboratories culture collection) were grown on Littman agar and Sabouraud-dextrose agar (SAB) (BBL Microbiology Systems) with 1% Tween 80 (Atlas Chemical) and incubated at 37°C for 7 days in culture flasks enclosed with cotton plugs. *P. ovale* was also cultured on yeast nitrogen base (BBL) with 1.5% agar

(YNBA) supplemented with the following: L- α -lecithin (1%) (Sigma Chemical Co.), oleic acid or triolein (1%) (Sigma Chemical Co.), human sebum (1%) collected by the method of Marples et al. (6), and SAB with human sebum (1%).

Sampling and analysis procedure. Preliminary studies indicated that a higher recovery of volatiles was obtained from a solid than a liquid culture medium. Therefore, in all experiments reported, solid media were used.

Single- or double-neck round-bottom flasks (250 ml) were fitted with a nitrogen inlet tube and an outlet tube which was attached to a stainless-steel tube (6 by 0.13 in. [15.2 by 0.32 cm]) containing 70 mg of Tenax (Applied Science Laboratories), an organic polymeric absorbant. The Tenax tubes were reused after heating to 280°C under nitrogen for 4 to 15 h. The headspace of the culture was swept with nitrogen at a flow rate of 90 ml/min for 17 h at 37°C. The collected volatiles were then backflushed with heating (220°C for 10 min) onto the first 15 cm of the GC column, which was cooled with dry ice. The volatiles were then separated and identified by combined GC-mass spectrometric analyses. The GC column (3.05 m by 2 mm) was Pyrex 20 M Carbowax on 80/100 Gas-Chrom Q programmed at 70°C (4 min), 70 to 220°C (4°C/min). The GC-mass spectrometric system was a Perkin-Elmer 990 gas chromatograph (The Perkin-Elmer Corp.) interfaced with a Watson-Biemann separator to a Hitachi/Perkin-Elmer RMU-6L mass spectrometer (13). The mass spectrometer conditions included: ionizing voltage of 70 eV; source temperature of 200°C; and the temperature of the GC-mass spectrometric interface at 260°C. The mass spectra were recorded on a B and F model 3006 oscillographic recorder and counted and interpreted manually. Individual components were identified by comparison of their fatty acid ethyl ester retention indices on a Carbowax column with those previously reported (12, 14), by comparison of retention times and mass spectral data with authentic samples, or by the reported mass spectral data (2, 7). Fatty acid ethyl ester values obtained for the γ -lactones

include: γ -hexa-, 10.6; γ -hepta-, 11.7; γ -octa-, 12.6; γ -nona-, 14.0; γ -deca-, 15.0; γ -undeca-, 16.1; and γ -dodeca-, 17.4. Uninoculated culture media, sterilized by either autoclaving or membrane filtration (Millipore Corp.), showed several volatile components including alkyl pyrazines, phenol, benzaldehyde, acetophenone, and furfural. Either olfactory examination or mass spectral analysis was necessary to confirm the presence of the lactones in cultures containing triolein because of the interfering contaminants in the triolein itself, some of which appeared to be alkyl phenols. Blank SAB cultures contained trace amounts of isopentanol and phenylethanol.

RESULTS

The odor profile obtained for *P. ovale* on lecithin-YNBA is shown in Fig. 1. The major odorants included a homologous series of γ -lactones. The presence of the lactones was readily confirmed by mass spectral analysis and GC retention times (fatty acid ethyl ester values) with either known compounds or reported data (2, 7, 12, 14). Increased sensitivity for detecting

lactones which were present in trace amounts was obtained by selective single-ion monitoring of the GC effluent at m/z 85, which is the base peak for γ -lactones, in combination with fatty acid ethyl ester retention indices.

All *Pityrosporum* cultures showed octa-, nona-, and γ -decalactones, with the last one as the major component (Table 1). Olfactory examination of the culture plates of *Pityrosporum* indicated an odor very similar to that of the γ -decalactone. Phenylethanol and γ -octalactone have similar retention times and, in most cases, the lactone appears as a shoulder on the larger phenylethanol peak. In some cultures, γ -undecalactone and γ -dodecalactone were also observed (Tables 1 and 2).

The volatiles which could be identified in the culture headspaces of *S. cerevisiae*, *R. rubra*, *T. glabrata*, and *C. albicans* on both SAB with Tween 80 and on Littman agar are shown in Table 3. None of the strains tested produced detectable amounts of lactones, but phenyl-

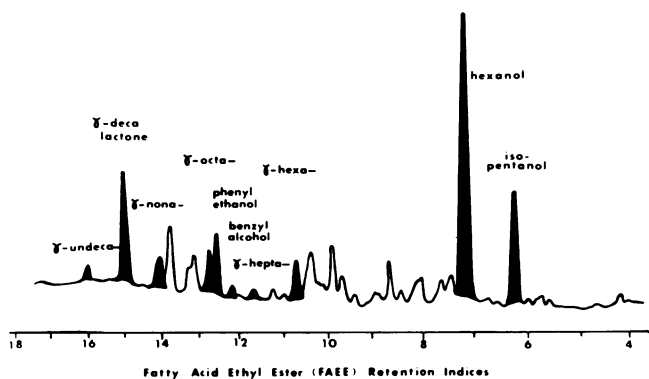


FIG. 1. Odor profile of *P. ovale*.

TABLE 1. Odor profile of *pityrosporum*^a

Compound	<i>P. ovale</i>		<i>P. orbiculare</i>		<i>P. canis</i>		<i>P. pachydermatis</i>	
	Littman ^b	SAB ^c	Littman	SAB	Littman	SAB	Littman	SAB
γ -Octalactone	30	— ^d	? ^e	10	6	0	2.5	—
γ -Nonalactone	7	1	3	6	5	0	1	1
γ -Decalactone	21	15	33	48	28	18	7	10
γ -Undecalactone	0	0	0	0	2	0	0	0
γ -Dodecalactone	0	3	19	0	5	0	0	3
Isopentanol	0	4,480	88	252	0	1,728	65	1,000
Hexanol	200	4	6	0	0	0	18	6
Benzyl alcohol	14	6	7	16	50	20	100	6
2-Phenylethanol	26	2,240	0	80	46	1,288	29	1,000

^a Relative GC peak intensities on 2×10^{-9} A with one-half of the material going to the mass spectrometer. Volatiles were concentrated on Tenax and transferred with heating to the gas chromatograph before analysis.

^b Cultured on Littman agar for 7 days.

^c Cultured on SAB with 1% Tween for 7 days.

^d —, Overlap of peak with phenylethanol peak.

^e ?, Presence uncertain.

ethanol and isopentanol were present in large amounts when grown on SAB with 1% Tween 80.

P. ovale, the most common yeast found on the human skin, was cultured on YNBA with individually added substrates to assess the requirements for lactone formation. No growth was observed on YNBA with added glycerol. The addition of lecithin resulted in the production of six different lactones, including γ -hexalactone and γ -heptalactone, which were not detected in the other culture media (Fig. 1 and Table 2). Lactones were also produced in the presence of added oleic acid, triolein, or human sebum (Table 2). The GC profiles of *P. ovale* on YNBA or SAB medium with added sebum were complicated by additional peaks from sebum itself, such as the food antioxidants butylated hydroxytoluene and ditertiarybutylhydroxyanisole (3). Slightly longer incubation time results in a scalp-like odor in these cultures. Headspace analysis shows the presence of short-chain ali-

phatic acids, lactones, and other unidentified components.

DISCUSSION

Yeasts of the genus *Pityrosporum*, when incubated on media containing lipid, emit a unique odor profile consisting of a homologous series of lactones. These lactones were identified by GC-mass spectrometry analysis of the culture headspace. Since other commonly identified yeast species cultured on lipid-containing media do not generate lactones, this profile may be a useful characteristic in the identification and differentiation of *Pityrosporum* from other yeast genera. The fact that these lactones are readily recognized as a canned-peach fruity odor makes it potentially useful for presumptive identification of these organisms.

Lactones have been reported as metabolites of other microorganisms. A soil fungus, *Trichoderma viride*, produces 6-pentyl-2-pyrone, a δ -lactone, as its major odorant (1). Another organism, *Ceratocystis moniliformis*, generates γ -decalactone when glycerol is used as a major substrate (4). Other microorganisms are known which reduce γ -keto acids to γ -hydroxy acids, which in turn cyclize to γ -lactones (9).

Phenylethanol and isopentanol are prominent components of most of the cultures tested on both media, but they have also been reported to be formed by other microorganisms (1, 10). In addition, compounds related to phenylethanol, such as styrene, phenyl acetaldehyde, and phenylethanol acetate, were found in some of the samples.

Our in vitro studies demonstrated that a lipid substrate is essential for both growth and lactone production by *P. ovale*. Human sebum provides sufficient nutrients for growth. When added to a medium such as SAB a marked increase in the lactone production occurs.

Since the *Pityrosporum* species, particularly *P. ovale*, are found as the predominant orga-

TABLE 2. Odor profile of *P. ovale* on selected media^a

Compound	Substrate			
	YNBA-lecithin	YNBA-oleic acid ^b	YNBA-sebum	SAB-sebum ^c
γ -Hexalactone	15	0	0	0
γ -Heptalactone	3	0	0	0
γ -Octalactone	20	3	11	— ^d
γ -Nonalactone	15	3	8	180
γ -Decalactone	42	—	0	340
γ -Undecalactone	8	0	0	33
γ -Dodecalactone	0	0	0	—
Isopentanol	45	80	0	210
Hexanol	280	70	0	0
Benzyl alcohol	4	0	10	120
2-Phenylethanol	24	0	—	5,760

^a Relative GC peak intensities on 2×10^{-9} A with one-half of the material going to the mass spectrometer. Volatiles were concentrated on Tenax and transferred with heating to the gas chromatograph before analysis.

^b Many additional peaks from oleic acid itself.

^c Fourteen-day culture; 34-h collection.

^d —, Overlap of peak with other components.

TABLE 3. Odor profile of other yeast genera^a

Compound	<i>R. rubra</i>		<i>C. albicans</i>		<i>T. glabrata</i>		<i>S. cerevisiae</i>	
	Littman ^b	SAB ^c	Littman	SAB	Littman	SAB	Littman	SAB
Lactones	0	0	0	0	0	0	0	0
Isopentanol	36	8,896	45	95	215	426	172	3,936
Hexanol	0	90	0	0	0	0	0	0
Benzyl alcohol	0	0	3	0	0	0	0	0
2-Phenylethanol	— ^d	192	364	4,000	156	704	572	269

^a Relative GC peak intensities on 2×10^{-9} amps with one-half of the material going to the mass spectrometer. Volatiles were concentrated on Tenax and transferred with heating to the gas chromatograph before analysis.

^b Cultured on Littman agar for 7 days.

^c Cultured on SAB with 1% Tween for 7 days.

^d —, Overlap of peak with culture contaminants.

nisms on the human scalp where there is abundant sebum and other nutrients, these lactones may play an important role in the formation of scalp odors. This is particularly suggested by the in vitro studies in which older cultures of *P. ovale* on SAB with added human sebum produced scalplike odor.

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