Formation of Extracellular C_{14}-C_{18} 2-d-Hydroxy Fatty Acids by Species of *Saccharomycopsis*

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Eighteen of 19 strains of *Saccharomycopsis fibuligera* and one of two strains of *S. capsularis* produced mixtures of C_{14}-C_{18} 2-d-hydroxy acids in liquid culture medium. The mixture of these acids showed antimicrobial activity against *Vibrio tyrogenus* but not against the other microorganisms tested. *Candida lactosa*, a recently described species, was shown to be an isolate of *S. fibuligera*.

*Saccharomycopsis* (*Endomycopsis*) *fibuligera* (Lindner) Klöcker is a well-known and widely distributed yeast frequently isolated from starchy substrates such as moist bread and Oriental fermented foods. Wickerham et al. (9) apparently were the first to demonstrate conclusively that *S. fibuligera* produces an extracellular amylase, and their finding has been put to such practical uses as the Symba yeast process in which agricultural and industrial processing wastes are degraded (3). Microscope observation of the culture medium from most strains of *S. fibuligera* reveals abundant needle-shaped crystals, but their presence and composition seem previously to have gone unreported. The crystals, commonly 3 to 5 μm long, are produced on a variety of media including malt extract (ME), yeast-maltose (YM), yeast morphology agar (8), and corn meal agar. Either extraction of the culture medium removed at least 75% of the crystals as shown by microscope observation. We have identified this crystalline material as a mixture of 2-d-hydroxy fatty acids and found it to have limited antibacterial activity.

The media and methods for identifying the yeast cultures were those of Wickerham (8), and the classification system was that proposed by van der Walt and Scott (4) and von Arx (1). The 2-d-hydroxy acids were ether-extracted from 4-day-old shake cultures grown in a liquid medium containing 5% Cerelose and 5% malt extract. None of these acids were detected in an ether extract of uninoculated culture medium. The crude ether extract of 2-d-hydroxy acids was first converted to methyl esters (diazo-methane) and then to trimethylsilyl derivatives (Regisil, Regis Co.) that were analyzed in a Packard gas chromatograph (model 562). The column (stainless steel, 122 cm by 2 mm inside diameter) was packed with 5% Apiezon L on Chromosorb W, and the temperature was maintained at 190 C. The optical rotatory dispersion measurements of the 2-hydroxy fatty acid mixture from NRRL Y-7170 show nearly the same negative dispersion curve as do those of 2-d-hydroxypalmitic acid, thus indicating a d-configuration. We have assumed the other strains examined also produce these 2-hydroxy fatty acids with a d-configuration.

Several samples of 2-d-hydroxy acid mixtures were tested by the paper disk method for antimicrobial activity against *Staphylococcus aureus* Rosenbach NRRL B-313, *Bordetella bronchiseptica* (Ferry) Moreno-López NRRL B-140, *Mycobacterium phlei* Lehmann and Neumann NRRL B-610, *Bacillus subtilis* Cohn NRRL B-3284, *Vibrio tyrogenus* (Flügge) Berg et al. NRRL B-1033, *Vibrio parahaemolyticus* (Fujino et al.) Sakazaki et al. NRRL B-4167, *Xanthomonas campestris* (Pammel) Dowson NRRL B-1459A, and *Candida albicans* (Robin) Berkhourt NRRL Y-477. Each 12.7-mm paper assay disk (no. 740 E, Schleicher & Schuell, Inc., Keene, N.H.) contained either 0.5 or 5.0 mg of the acid mixture, which was added to the disks as a chloroform solution. Other disks were pretreated with chloroform alone, ether extract from uninoculated culture medium, uninoculated culture medium, and inoculated culture medium that was heated for 15 min at 100 C to kill the cells. The disks were placed on the surface of pour plates (90 mm) containing 20 ml of agar with either 5 × 10^8 bacteria or 5 × 10^9 yeast per ml. *Vibrio parahaemolyticus* was grown in nutrient agar (Difco) with 3% NaCl at 37 C, but the other microorganisms were tested in TGY agar (0.5% yeast extract, 0.5% tryptone, 0.1% glucose, 0.1%...
K$_2$HPO$_4$, and 2.0% agar) at 28 C. Duplicate plates were prepared with the agar set at pH 6.0 and 7.0.

Of the 19 strains of *S. fibuligera* examined, only one did not produce a mixture of 2-d-hydroxy acids (Table 1). The material in the culture medium of this isolate, NRRL Y-6720, was a mixture of low-molecular-weight, free fatty acids that was not further characterized. Quantitation through use of a silicic acid (BioSil A, 100 to 200 mesh) column showed 81.5% of the crude ether extract from the culture medium of NRRL Y-7170 to consist of 2-d-hydroxy acids, and the percentage composition given in Table 1 refers to this fraction. For nearly all strains, the major component in the mixture was 2-D-hydroxyhexadecanoic acid, with the C$_{18}$ homologue being next most abundant. Only two of the cultures produced detectable amounts of 2-D-hydroxypentadecanoic acid.

Analysis of the culture medium from two strains of the apparently related species *S. capsularis* Schöning revealed one to produce a mixture of 2-d-hydroxy acids, whereas the other did not.

Recently NRRL Y-7170 was described as *Candida lactosa* Dwidjoseputro (2), but in our examination it readily sporulated and was indistinguishable from other isolates of *S. fibuligera*.

In tests for antimicrobial activity, *Vibrio tyrogenes* NRRL B-1033 was inhibited by the 2-d-hydroxy acid mixture from NRRL Y-7170 at both the 0.5-mg (4-mm zone) and 5.0-mg (9-mm zone) levels at pH 6 but not at pH 7. There was no inhibition by disks with uninoculated culture medium, ether extract of uninoculated culture medium, or inoculated culture medium with heat-killed cells. In this last treatment, the concentration of 2-d-hydroxy acids apparently was too low to cause inhibition. The other microorganisms tested were unaffected by any of the treatments.

Vesonder et al. (5) isolated free 2-d-hydroxy acids from the culture medium of *Hansenula sydowiorum* Scott et van der Walt and pointed

Table 1. Occurrence of 2-d-hydroxy acids in culture medium from *Saccharomycopsis fibuligera* and *S. capsularis*

| Species and NRRL no. | Source of cultures | 2-d-Hydroxy acid mixture Presence | Mg of ether extract per liter of culture medium |
|---------------------|-------------------|----------------------------------|------------------------------------------------|
| **S. fibuligera**   |                   |                                  |                                                |
| Y-3                 | Probably from peh-yüeh | + 3.15 0.00 41.62 1.36 6.14 | 40                                             |
| Y-25                | Unknown            | + 19.87 0.00 18.34 13.43 6.04 | 40                                             |
| Y-1062              | Wheat flour        | + 4.20 0.00 70.88 0.94 4.67   | 75                                             |
| Y-2385              | Compressed yeast   | + 3.12 0.00 38.09 1.60 11.66  | 100                                            |
| Y-2386              | Unknown            | + 1.44 0.15 69.88 1.19 8.69   | 50                                             |
| Y-2387              | Unknown            | + 1.89 0.18 6.55 0.74 29.41   | 110                                            |
| Y-2388              | Bread (type strain)| + 1.70 0.00 24.11 0.79 11.16  | 100                                            |
| Y-6720              | Peh-yüeh           | _ b                                | 100                                             |
| Y-7061              | Chica starter      | + 0.54 0.00 54.63 0.00 5.93   | 190                                            |
| Y-7145              | Ragi               | + 1.78 0.00 56.43 1.63 8.30   | 115                                            |
| Y-7170              | Ragi-tape          | + 0.00 0.00 63.78 2.51 13.38  | 108                                            |
| Y-7221              | Japanese tempeh    | + c                                | 105                                            |
| Y-7324              | Tibetan beer starter | + c                          | 175                                            |
| Y-7325              | Tibetan beer starter | + c                       | 175                                            |
| Y-7326              | Tibetan beer starter | + c                          | 190                                            |
| Y-7464              | Irradiated bread   | + 0.00 0.00 88.77 0.38 3.62   | 67                                             |
| Y-7488              | Ragi               | + 0.46 0.00 39.52 1.22 8.21   | 69                                             |
| Y-7489              | Ragi               | + 0.00 0.00 88.77 0.38 3.60   | 90                                             |
| Y-7490              | Tape               | + 0.76 0.00 27.56 0.00 3.68   | 104                                            |
| **S. capsularis**   |                   |                                  |                                                |
| Y-7486              | Unknown            | + e                                |                                                |
| Y-7487              | Pollen, *Xylocopa caffra* | + e                         |                                                |

* Percent composition relative to total quantity of compounds detected under gas-liquid chromatography conditions used; this fraction assayed was estimated to represent 70 to 80% of the crude ether extract from the culture medium.

* Crystals present, a mixture of low-molecular-weight free fatty acids detected.

* Composition not determined.
out that this was the first time these acids were detected free in the culture medium of a yeast. Previously, the acids had been found as constituents of more complex molecules in other microorganisms. The composition of the 2-d-hydroxy acid fraction from *H. sydowiorum* (0.7% C\textsubscript{14}, 4.2% C\textsubscript{15}, 78.5% C\textsubscript{16}, 11.4% C\textsubscript{17}, 5.0% C\textsubscript{18}) is similar to that of *S. fibuligera* but somewhat higher in the C\textsubscript{15} component (5, and unpublished data). We tested a sample of this 2-d-hydroxy acid mixture and found it also inhibitory to *V. tyroenous*.

The taxonomic significance of the occurrence of these acids cannot be adequately evaluated at this time because so few species have been examined. Since antibacterial activity was observed against only one strain in our tests, the possibility that these compounds might aid in establishing a species in a particular habitat or might have some preservative action in Oriental fermented foods seems unlikely. Wang et al. (6, 7) had previously reported that several species of *Rhizopus* isolated from these same fermented foods possessed antibacterial activity.

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**LITERATURE CITED**

1. Arx, J. A. von. 1972. On *Endomyces, Endomycopsis* and related yeast-like fungi. Antonie van Leeuwenhoek J. Microbiol. Serol. 38:289-309.
2. Dwijoseputro, D., and F. T. Wolf. 1970. Microbiological studies of Indonesian fermented foodstuffs. Mycopathol. Mycol. Appl. 41:211-222.
3. Jarl, K. 1969. Symba yeast process. Food Technol. 23:1009-1012.
4. van der Walt, J. P., and D. B. Scott. 1971. The yeast genus *Saccharomycopsis* Schöning. Mycopathol. Mycol. Appl. 43:279-288.
5. Vesonder, R. F., F. H. Stodola, W. K. Rohwedder, and D. B. Scott. 1970. 2-d-Hydroxyhexadecanoic acid: a metabolic product of the yeast *Hansenula sydowiorum*. Can. J. Chem. 48:1985-1986.
6. Wang, H. L., J. J. Ellis, and C. W. Hesseltine. 1972. Antibacterial activity produced by molds commonly used in Oriental food fermentations. Mycologia 64:215-221.
7. Wang, H. L., D. I. Ruttle, and C. W. Hesseltine. 1969. Antibacterial compound from a soybean product fermented by *Rhizopus oligosporus*. Proc. Soc. Exp. Biol. 131:579-583.
8. Wickerham, L. J. 1951. Taxonomy of yeasts. U.S. Dept. Agr. Bull. no. 1029:1-56.
9. Wickerham, L. J., L. B. Lockwood, O. G. Pettijohn, and G. E. Ward. 1944. Starch hydrolysis and fermentation by the yeast *Endomycopsis fibuliger*. J. Bacteriol. 48:413-427.