Evidence of Strecker Aldehyde Excretion by Yeast in Cold Contact Fermentations

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A medium containing labeled leucine-d_{10} has been used to show that *Saccharomyces cerevisiae* was able to produce deuterated 3-methylbutanal in a cold contact fermentation. Whereas residual unreduced Strecker aldehydes bound to polyphenols were considered until now as the main defect of alcohol-free beers, yeast emerges from this work as an additional source of worty flavor. When the same experiment was conducted without leucine, 3-methylbutanal excretion also occurred, indicating that valine could be used by yeast as precursor.

Keywords: *Strecker aldehyde; Saccharomyces cerevisiae; amino acid; excretion; cold contact process*

INTRODUCTION

Strecker aldehyde monitoring in cold contact fermentation has revealed fast initial enzymatic reduction that quickly slows. This leads to residual concentrations amounting to as much as 40% of the concentration in the wort (Perpète and Collin, 1999a,b). These aldehydes that have escaped reduction appear to be bound to polyphenols, mainly when the temperature is low (Perpète and Collin, 2000; Jung et al., 2000). Although the cold contact process is usually stopped after a few hours, it seemed interesting to check what would happen if it were extended to 24 h. At 0 °C, yeast metabolism is of course slow, leading to very low ethanol excretion (Schur, 1983; van Iersel et al., 1995). On the other hand, as described here, aldose reductase activity appears to be significant. What about other enzymatic activities such as those involved in the Ehrlich pathway?

The aim of this paper was to monitor aldehydes through a cold contact process in which a labeled amino acid was added to the fermentation medium.

MATERIALS AND METHODS

Reagents. 3-Methylbutanal (98%) was from Janssen Chimica (Geel, Belgium). Leucine-2,3,3,4,5,5,5,6,6,6-d_{10} (Figure 1) was from Aldrich (Bornem, Belgium).

Strains. Bottom fermentation *Saccharomyces cerevisiae* MUCL28365 was provided by the MUCL collection of the Université Catholique de Louvain (Louvain-la-Neuve, Belgium).

Culture Media. Cultures were grown in YPS medium (1% yeast extract, 0.5% peptone, 10% sucrose) at 28 °C on a rotary shaker and collected in the exponential phase. Propagation was carried out in two steps with a pitching rate of 10^7 cells/mL each time. After collection and washing, the yeast was pitched at the same level in a 50 mM phosphate buffer containing 10% sucrose. This simple medium was chosen to avoid any additional source of leucine and to minimize 3-methylbutanal concentration. In a first experiment, 2205 ppm of the usual wort amino acids was added (Table 1). In a second experiment, leucine was replaced by 160 ppm of deuterated leucine. For the third cold contact fermentation, no leucine at all was added. In all media, small amounts of 3-methylbutanal (~30 ppb) appeared from the yeast pitching.

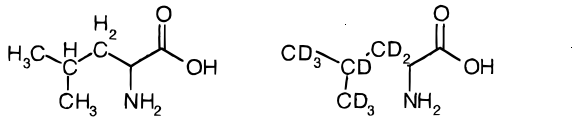

Figure 1. Structures of leucine and leucine-2,3,3,4,5,5,5,6,6,6-d_{10}.

Table 1. Amino Acid Composition of the Model Solution

amino acid	concn (ppm)	amino acid	concn (ppm)
proline	350	lysine	95
γ-aminobutyric acid	350	isoleucine	85
leucine	160	serine	70
arginine	150	glutamic acid	60
phenylalanine	150	histidine	60
threonine	150	aspartic acid	55
valine	130	tryptophan	45
alanine	120	glycine	40
tyrosine	100	methionine	35
total			2205

Cold Contact Sampling. Each sample was centrifuged at 4 °C to remove yeast and immediately frozen in liquid nitrogen.

Dynamic Headspace Gas Chromatography: FID Analytical Conditions. A Hewlett-Packard model 5890 gas chromatograph equipped with a Chrompack purge and trap injector, a flame ionization detector, and a Shimadzu CR3A integrator was used. Samples were injected into the chromatographic column in three steps as follows: (1) The trap was precooled (CPSIL 8 CB capillary column, 0.53 mm i.d.; film thickness = 5 μm). The trap was cooled at −95 °C for 2 min in a stream of liquid nitrogen. (2) The sample was purged. The temperature of the purge vessel was set at 50 °C. The sample was purged with helium gas (12 mL/min) for 15 min. The gas stream was passed through a condenser kept at −15 °C by means of a cryostat (Colora WK 15) to remove water vapor and then through an oven at 200 °C. The volatiles were finally concentrated in the cold trap maintained at −95 °C (liquid nitrogen). (3) The volatiles were desorbed. Cooling was stopped, and the surrounding metal capillary was immediately heated to 220 °C for 5 min. The carrier gas swept the trapped compounds into the analytical column. Analysis of samples was carried out on a 50 m × 0.32 mm, wall-coated, open tubular

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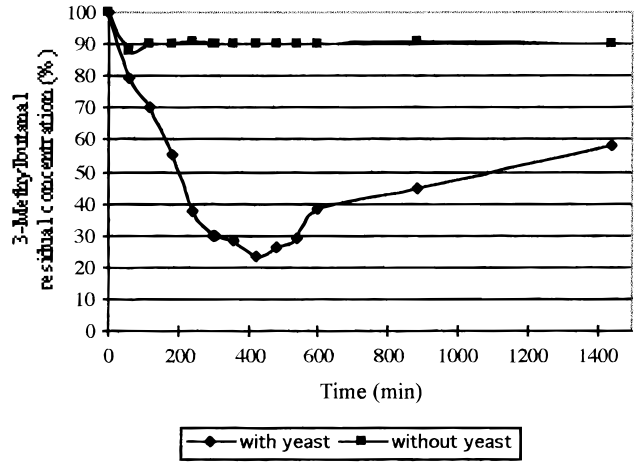

Figure 2. Evolution of 3-methylbutanal (percent of the initial concentration, 100% was 2.1×10^7 μV·s) in a 12 °P wort in cold contact process conditions (pitching 10^7 cells/mL).

(WCOT) CPSil5 CB (Chrompack, Antwerpen, Belgium) capillary column (film thickness = 1.2 μm). The oven temperature, initially kept at 36 °C for 15 min, was programmed to rise from 36 to 120 °C at 5 °C/min and then to 200 °C at 10 °C/min, remaining at the maximum temperature for 10 min thereafter. Helium carrier gas was used at a flow rate of 1.0 mL/min. Injection and detection temperatures were 200 and 220 °C, respectively. All analyses were done in duplicate. The assessment of the technique reproducibility has been previously described (coefficients of variation <10% for five analyses of the same standard mixture; Collin et al., 1993).

Gas Chromatography−Mass Spectrometry Conditions. The column was directly connected to an HP 5988 quadrupole mass spectrometer. Chromatographic conditions were the same as those used for FID detection except for a higher gas flow due to the 10^{-7} Torr vacuum applied in the detector. In that case, unfortunately, labeled and unlabeled deuterated 3-methylbutanal were no longer separated. Electron impact mass spectra were recorded at 70 eV (filament current = 300 mA; electron multiplier voltage = 2700 V; scan rate = 4 s^{-1}; *m/z* range = 40−100). Spectral recording throughout elution was automatic using HP59970C software. In the single-ion monitoring mode, *m/z* 44 and 58 were selected for the nonlabeled aldehyde, whereas *m/z* 48 and 64 were chosen for 3-methylbutanal-d_{10}.

RESULTS AND DISCUSSION

A first cold fermentation with and without yeast was carried out for 24 h on a 12 °P wort enhanced with 300 ppb of 3-methylbutanal (Figure 2). Although a typical reduction pattern was observed during the first 500 min (Debourg et al., 1994; Perpète and Collin, 1999a,b), the 3-methylbutanal level increased by 31% between 7 and 24 h of fermentation.

Release of carbonyl compounds from a chemically adsorbed fraction, for instance, polyphenol adducts (Perpète and Collin, 1999b), seems to be excluded because no aldehyde release was detected in the absence of yeast. To see whether aldehydes might be formed by yeast via the Ehrlich pathway and then excreted (Oshita et al., 1995), a cold contact experiment was started in which leucine was replaced with labeled leucine-d_{10}. We figured that if yeast could excrete a leucine-derived Strecker aldehyde in a cold fermentation, labeled 3-methylbutanal-d_{10} should be observed. Figure 3 shows the mass spectrum of the labeled aldehyde (obtained at 100 °C after 1 h from 10 mg of leucine-d_{10} and 20 mg of glucose in 0.5 mL of water). This standard was characterized, under our GC-FID conditions, by a retention time 0.6 min lower than that of the unlabeled compound.

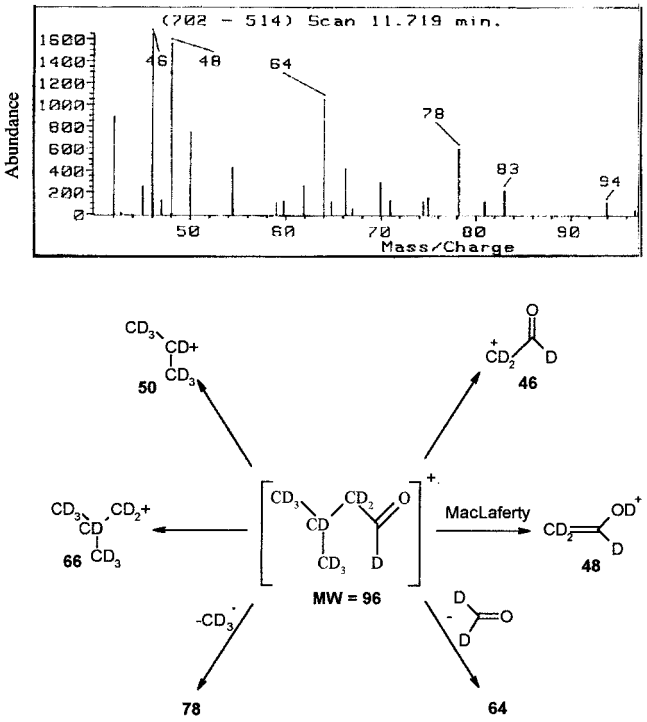

Figure 3. Mass spectrum of 3-methylbutanal-d_{10} obtained by leucine-d_{10} pyrolysis and tentative identification of ions. Unexplained fragments (except *m/z* 42 and 54) come from the noise.

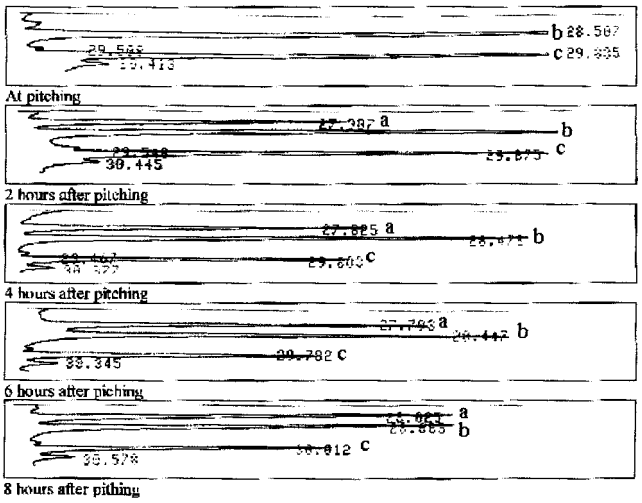

Figure 4. Dynamic headspace chromatograms through the cold contact fermentation after 0, 2, 4, 6, and 8 h in the presence of leucine-d_{10} (a, deuterated 3-methylbutanal; b, 3-methylbutanal; c, 2-methylbutanal).

A nice chromatographic separation appeared through the cold fermentation, 3-methylbutanal-d_{10} (a in Figure 4) being detected after 2 h. Its identity was confirmed by mass spectrometry in the single-ion monitoring mode.

Figure 5 shows how concentrations of labeled and unlabeled 3-methylbutanal evolved through the cold fermentation, as compared to a control with unlabeled leucine.

From this experiment, it appeared that yeast can indeed produce aldehydes from amino acids (Ehrlich

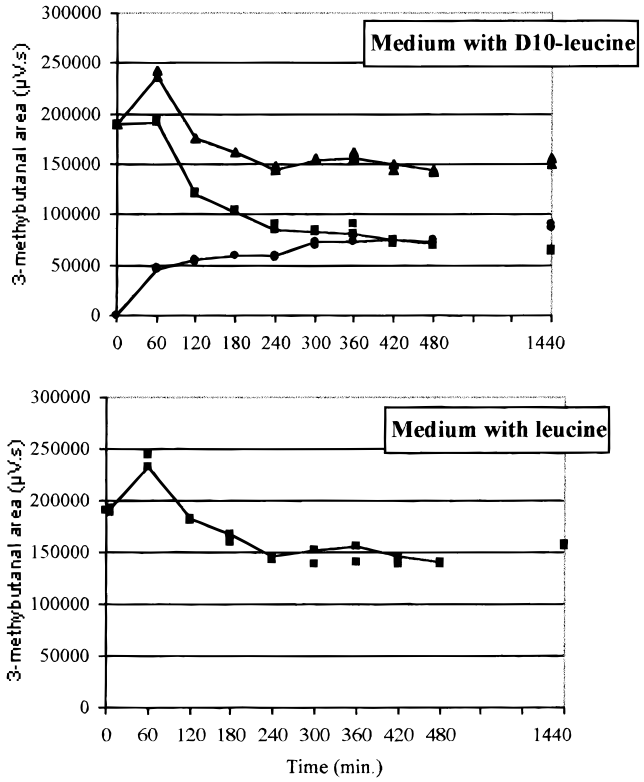

Figure 5. Evolution of 3-methylbutanal area in media containing either labeled or unlabeled leucine-d_{10} (cold contact fermentation, pitching at 10^7 cells/mL; ●, deuterated; ■, undeuterated; ▲, total). All experiments were done in duplicates.

pathway) a few hours after pitching, despite the very low temperature. When for each point in time the amounts of deuterated and undeuterated 3-methylbutanal were summed, the sum was found to evolve, as is logical, like the concentration of 3-methylbutanal in the control.

The 3-methylbutanal level appeared to have increased much earlier in the laboratory medium than in the industrial wort (Figure 2). Aldehyde synthesis and excretion are obviously more easily detected in this very poor medium (similar excretion and reduction scales). On the other hand, only wort allowed excretion at the end of a cold contact experiment (between 500 and 1400 min).

Surprisingly, when the same experiment was conducted without leucine, the same pattern was again obtained (Figure 6). Although only one sampling took place between 0 and 120 min, we suspect that another amino acid (valine probably; Walker, 1998) might induce 3-methylbutanal synthesis in this case. This experiment gives a glimpse of how difficult it will be to analyze the Ehrlich pathway by gene disruption.

CONCLUSIONS

Using a medium containing labeled leucine-d_{10}, we have shown that *Saccharomyces cerevisiae* can produce 3-methylbutanal from this amino acid in a cold contact fermentation. Until now, residual polyphenol-bound Strecker aldehydes have been considered to be the main

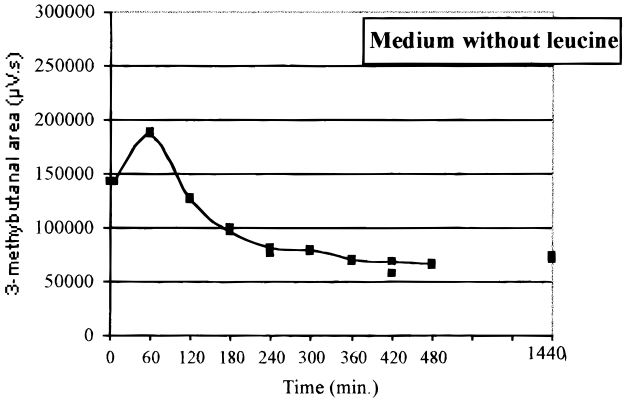

Figure 6. Evolution of 3-methylbutanal area in a leucine-free medium (cold contact fermentation, pitching at 10^7 cells/mL). All experiments were done in duplicates.

defect of alcohol-free beers, but now yeast emerges from this work as an additional source of the worty flavor. Aldehyde excretion, however, seems to depend greatly on the medium composition. The cold contact time should thus be optimized for each set of industrial conditions so as to avoid abundant synthesis and excretion of aldehydes.

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